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A TWO YEARS' SURVEY OF POPULATION DYNAMICS IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

Natural populations of Drosophila species have been shown to contain numerous autosomes producing a lethal or deleterious effect, when made homozygous. If the selective value of these autosomes depends mainly on their action in the homozygous state, their concentration should be reduced by inbreeding, as assumed by Dubinin (1946), while large panmictic populations should accumulate a high percentage of them. The amount of lethal and deleterious autosomes in a population might thus be considered as a "yardstick" of population size (Ives, 1945). If, on the other hand, the effect of these chromosomes in the heterozygous state modifies their selective value to an appreciable extent (Wright *et al.*, 1942; Stern *et al.*, 1952; Cordeiro, 1952), the simple and direct relationship between lethal content and population size may not exist. Pavan *et al.* (1951) found little differentiation between populations of *D. willistoni* from various parts of Brazil with regard to deleterious chromosome content, although the same populations differed widely in the incidence of inversions. But Townsend (1952) showed that marginal populations of this species contain fewer recessive mutants than those of central Brazil. A striking difference in deleterious chromosome content of *D. pseudoobscura* inhabiting Central America and California, respectively, was demonstrated by Dobzhansky (1939). A comparison between the data of Ives (1945, 1954), Dubinin (1946) and Goldschmidt (1951) suggests a similar divergence between the populations of *D. melanogaster* in the United States, Russia and the Near East. Moreover, regional differences within the boundaries of the United States have been repeatedly observed by Ives (1945, 1954). There exists, thus, a certain amount of evidence for the geographical variation in deleterious gene content, but further proof of the correlation between this variable and between population size is desirable.

Dubinin (1946) and Ives (1945, 1954) claim that this correlation should become manifest by population differences in time as well as in space. According to Dubinin the annual reduction of the population during the unfavorable season should be reflected in a decrease of lethal genes, while Ives stresses the possibility of long range changes in recessive gene content as a result of population growth or dwindling over the years.

The present investigation was planned to check the deleterious gene incidence in a *D. melanogaster* population during two years and thus to test for the "Dubinin cycle." At the same time, it was intended to furnish further data on the population structure of this species in Israel for comparison with the results obtained by other workers in Russia and the United States.

Material

The six samples of flies utilized in the tests were all collected in the orchards of Qiryat'Anavim (625 m above sea level), a communal settlement in a rural area of the Judean Hills, and in a small oak wood 1 km distant from the settlement. The climate of the area is characterized by the data of table I. The district has a fruit growing tradition of long standing, and the settlement itself has been in existence for over 30 years. There are no citrus groves in the whole neighbourhood.

Milk bottles with and without paper funnels provided with banana-yeast bait (or with melon or guava when bananas were scarce) were set up in the shade of bushes, rocks and trees and collected after half a day to three days. While during population peaks the bottles would swarm with flies after a few hours, longer periods of exposure were required at more unfavourable seasons. In spite of the variations in exposure time and the tendency of the flies to aggregate in particular spots, probably in relation to the ripening of the different kinds of fruit, the number of flies per bottle may be considered a very rough indicator of population activity at the different times of the year (table II). No flies could be collected during the winter 1951/2, but in the beginning of the rainy season of 1952/3 prolonged visits to the collecting site assembled a small sample of flies. Table II indicates that later in this year there was a marked reduction in population activity. Other species collected in the bottles will be considered in a separate publication.

Viability of Second Chromosomes

Wild males collected at the site or sons of wild females allowed to oviposit in "creamers" were utilized for the well-known series of crosses with the *Cy L/Pm* stock (Wallace, 1950), producing in

Table I. *Temperature, relative humidity and rainfall for Ma'ale haHamisha* in 1951, 1952 and 1953. Compiled from monthly weather reports (State of Israel, Meteorological service)*

	Temperature means °C.									Relative humidity daily mean %			Rainfall mm		
	Daily			Maximum			Minimum								
	'51	'52	'53	'51	'52	'53	'51	'52	'53	'51	'52	'53	'51	'52	'53
Jan.	11.2	9.9	9.9	15.0	12.9	13.1	7.5	6.9	6.7	56	70	61	104	162	130
Feb.	10.5	10.9	10.9	13.7	14.3	14.1	7.3	7.5	7.7	71	69	68	158	150	189
March	14.9	11.6	9.0	19.2	15.4	12.2	10.6	7.9	5.8	56	69	72	61	120	325
April	16.9	16.6	15.3	21.1	21.2	19.5	12.7	11.9	11.1	56	55	59	14	11	6
May	20.4	20.2	19.5	25.5	26.0	24.9	15.2	14.5	14.1	48	49	50	—	—	—
June	22.0	22.0	21.8	27.1	27.7	26.9	16.9	16.2	16.6	58	58	60	—	—	—
July	23.8	23.0	24.6	28.8	28.6	29.4	18.7	17.4	19.7	61	67	59	—	—	—
Aug.	24.6	25.2	23.4	29.8	31.3	28.7	19.5	19.7	18.2	59	61	69	—	—	—
Sept.	23.4	24.9	22.0	28.2	30.2	27.3	18.5	19.6	16.6	66	62	67	13	—	—
Oct.	19.8	21.4	20.3	24.2	25.8	25.2	15.3	17.0	15.4	65	61	59	18	19	—
Nov.	16.0	16.0	12.8	19.8	19.5	16.2	12.2	12.5	9.4	65	57	74	60	32	204
Dec.	9.0	14.2	9.0	11.5	17.5	12.1	6.6	10.8	5.8	78	56	68	429	42	162

* Ma'ale haHamisha is situated about 2 km NW from Drosophila collecting site. Height 810 m above sea-level.

the third generation *Cy L* flies heterozygous for one wild second chromosome and unmarked flies homozygous for the same chromosome. Five to seven pairs of second generation *Cy L* males and females were utilized for the test cultures. These parents were transferred twice, at intervals of three days, to fresh culture bottles. The culture medium was cornmeal-molasses-agar and the bottles were kept at 25 ± 1° C. In Spring 1953 we were unable to protect certain portions of the sample (88 chromosomes) from the rising heat. This section was subjected to a separate analysis and showed excellent agreement with the remainder of the sample, with which it was therefore pooled.

Each bottle was counted three times, the last count being made on the eighteenth or nineteenth day, i.e., before the eclosion of the next generation. In cases, where the first two cultures pertaining to a particular chromosome had already yielded over 500 flies before the emergence of the test generation in the third bottle, this last culture was discarded. The percentage of the unmarked flies in the total yield of the bottles scored was calculated for each wild chromosome and served as the index for the classification of the chromosomes according to viability (table III).

In the Spring sample of 1953, no exact analysis of the classes comprising the chromosomes with a yield above 16.66% unmarked flies was attempted. In this sample a chromosome was classified above the sublethal level, if its first count, scored to the end, yielded over 25% unmarked flies among over 150, and counts for this chromosome were then discontinued. If this relationship was not established after the first count, scoring was continued to the second, and if necessary to the third count. When the first culture, in repeated counts, yielded less than 25% unmarked flies, the second and if necessary the third bottle were counted to the end. Since in the majority of cultures the unmarked flies hatch later than

TABLE II. *Collection records of* Drosophila melanogaster *Males*

Date		No. of visits made	Total no. of bottles	Males of *D. melanogaster* trapped	
Year	Month			Total flies	Flies/ bottle
1951	October	1	34	256	7.53
1952	January*	2	20	—	—
1952	March*	4	43	5	0.12
1952	April	3	36	16	0.44
1952	May	1	16	355	22.19
1952	July	2	37	150	4.05
1952	August	1	29	52	1.79
1952	October	1	26	728	28.00
1953	January	5	79	63	0.80
1953	February*	1	4	10	2.50
1953	April*	1	20	1	0.05
1953	May	4	53	270	5.09

* Not included in experimental samples.

the *Cy L* type (a similar tendency was described for homozygotes of *D. willistoni* and of *D. pseudoobscura* as compared with the heterozygotes carrying the markers, cf. Pavan *et al.*, 1951), we believe that the margin of safety was sufficient to ensure correct classification above and below the 16.66% level, and that we could not have missed a single chromosome of the 0–3.33% class in this fashion.

The distribution of the viability of second chromosomes is very similar in the six samples investigated. This becomes clear on inspection of table III, of figure 1 and on examination of the χ^2 values for group differences between the samples (table IV). There is no significant difference between the samples for any of the viability classes, nor for the lethal and semilethal or for the "normal" ($> 26.66\%$) classes combined. The agreement is particularly striking for the latter group, which comprises almost exactly one half of the chromosomes of each sample.

If there was a "Dubinin cycle" in our population, we should register fluctuations in lethal gene content in conformance with changes in the size of the breeding population. We have no direct infor-

TABLE III. *Viabilities of homozygotes for second chromosomes in six samples from Qiryat 'Anavim. Viabilities are expressed as percentage of unmarked flies hatching in test cultures*

	Autumn 1951 Chromosomes		Spring 1952 Chromosomes		Summer 1952 Chromosomes		Autumn 1952 Chromosomes		Winter 1953 Chromosomes		Spring 1953 Chromosomes		Total Chromosomes	
Class	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0 – 3.33	45	30.61±3.80	76	30.28±2.90	59	28.37±3.13	96	33.57±2.79	35	37.23±4.99	82	34.75±3.10	393	32.16±1.34
3.34– 6.66	3	2.04±1.17	3	1.20±0.69	5	2.40±1.06	7	2.45±0.91	2	2.13±1.49	4	1.69±0.84	24	1.96±0.40
6.67– 9.99	3	2.04±1.17	6	2.39±0.96	2	0.96±0.68	7	2.45±0.91	—		4	1.69±0.84	22	1.80±0.38
10.00–13.33	4	2.72±1.34	1	0.40±0.13	5	2.40±1.06	4	1.40±0.69	1	1.06±1.06	3	1.27±0.73	18	1.47±0.34
13.34–16.66	4	2.72±1.34	1	0.40±0.13	2	0.96±0.68	7	2.45±0.91	1	1.06±1.06	6	2.54±1.02	21	1.72±0.37
16.67–19.99	3	2.04±1.17	13	5.18±1.40	6	2.88±1.16	7	2.45±0.91	2	2.13±1.49				
20.00–23.33	4	2.72±1.34	6	2.39±0.96	8	3.85±1.34	9	3.15±1.03	1	1.06±1.06				
23.34–26.66	7	4.76±1.76	18	7.17±1.63	11	5.29±1.55	9	3.15±1.03	5	5.32±2.31				
26.67–29.99	12	8.13±2.25	42	16.73±2.36	27	12.98±2.33	32	11.19±1.86	8	8.51±2.88				
30.00–33.33	29	19.73±3.28	57	22.71±2.64	54	25.96±3.04	60	20.98±2.41	25	26.60±4.56	137	58.05±3.21	744	60.88±1.40
33.34–36.66	27	18.37±3.19	24	9.56±1.86	28	13.46±2.36	36	12.59±1.96	14	14.89±3.67				
36.67–39.99	5	3.40±1.49	4	1.59±0.79	1	0.48±0.48	11	3.85±1.14	—					
40.00–43.33	—		—		—		1	0.35±0.35	—					
>43.33	1	0.68±0.68	—		—		—		—					
Total	147		251		208		286		94		236		1222	
Visibles	7		8		14		26		6		15		76	
Average viability above 16.66%		31.13		29.30		30.01		30.64		30.55		—		30.20

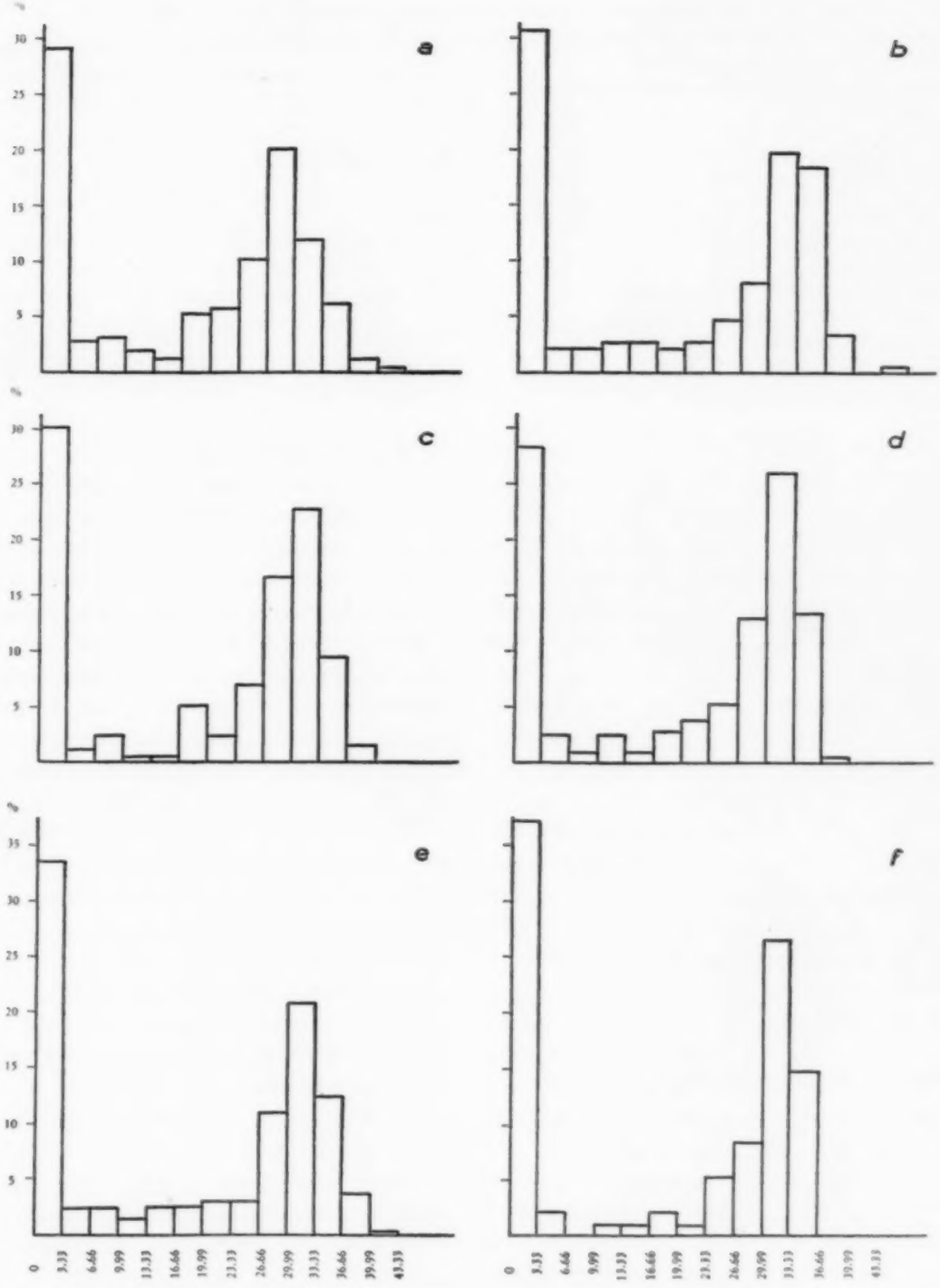

FIG. 1. Distribution of chromosome viabilities in six samples of *D. melanogaster* from Israel. The height of each column represents the percentage of chromosomes in a viability class. a—Autumn 1950, Jerusalem, b—Autumn 1951, c—Spring 1952, d—Summer 1952, e—Autumn 1952, f—Winter 1953 (b-f from Qiryat 'Anavim).

mation on the latter value, but from table II we may gather that a drastic reduction of the population can only occur in winter, during the period of minimum activity, if at all. Thus, there should be an increase in lethals and semilethals during the warm months and a drop in winter. A "tendency" to such an effect might be seen in the rise of the lethal class (0 – 3.33%), occurring from Summer

TABLE IV. *Chi-square and probability values for various samples and classes*

Samples compared	Classes	Chi-square	d.f.	p
Qiryat 'Anavim				
6 samples	0– 3.33%	4.03	5	0.7 –0.5
6 samples	0–16.66%	5.81	5	0.5 –0.3
Qiryat 'Anavim				
5 samples	3.34–26.66%	2.20	4	0.7 –0.5
5 samples	>26.66%	0.76	4	0.95–0.90
Qiryat 'Anavim				
3 samples 1951/2 & 3 samples 1952/3	0– 3.33%	3.33	1	0.1 –0.05
Jerusalem 1950 & Qiryat 'Anavim				
6 samples pooled	0– 3.33%	0.81	1	0.5 –0.3
6 samples pooled	0–16.66%	0.02	1	0.9 –0.8

1952 to Mid-Winter 1953. But not even the difference between the two extreme values ($28.37 \pm 3.13\%$ and $37.23 \pm 4.99\%$) is significant. The χ^2 for this difference amounts to 2.374 for one degree of freedom, corresponding to a probability between 0.2 and 0.1. Since the rise in the lethal class from Autumn 1952 till Winter 1953 (January) is accompanied with a drop in the combined lethal and semilethal classes at the same period, the "effect" seems all the more obviously spurious. The possibility of a second, though less marked, reduction in population size during the hot summer months cannot be excluded (cf. table II) and this may add further complication to the dynamics of the seasonal cycle.

The present survey can hardly be expected to reveal a "long-range" change in lethal incidence, since a period of only two years has been covered. If the first three and the last three samples are pooled (table IV), drawing the line rather arbitrarily between Summer and Autumn 1952, we obtain a difference of 4.87%, but even this remains just below the 5% significance level.

Figure 1 and table IV may also be consulted for a comparison of the sample from Israel analyzed by Goldschmidt (1951), which consisted mostly of chromosomes collected in Jerusalem in Autumn 1950, with the combined data of the present six samples. The agreement is, on the whole, very good. However, there exists one clear-cut difference: The right hand peak of the histogram of 1950 coincides with the 26.6–29.9% class, while each of the five complete histograms of the present investigation has a maximum at 30.0–33.3%. The difference between the sizes of these two classes in material originating from areas about 12 kms distant from one another is highly significant (χ^2 for one degree of freedom equals 20.6). Moreover, the classes above 33.3% are larger in the series from Qiryat 'Anavim than in the sample from 1950, and this apparent superiority of the normal homozygotes for the chromosomes of the present investigation is expressed, once again, in the value for the average viability of all chromosomes above the semilethal range, which amounts to 30.20% in the first five samples from Qiryat 'Anavim, whereas the corresponding figure for the 1950 collection is as low as 27.98%. In view of the general similarity between the two sets of data, it appears hardly warranted to interpret this divergence as an indication of a true difference in population structure. The test in 1950 was carried out in a different laboratory and under different culture conditions. Dobzhansky and Wallace (1953) and Wallace and Madden (1953) have shown that the viabilities of homozygotes are characterized by large variances, indicating a much more sensitive response to varying culture conditions in homozygous than in heterozygous individuals. It is also possible that the relative viability of the *Cy L* type, which in

1950 was tested by Dr. B. Wallace at Cold Spring Harbor, may have changed in our laboratory, under constant selection for good expression of the *Cy* and the *L* character.

Visible Mutations

The detection of mutations affecting visible characters was of course subjected to the personal errors of our team, although the six to nine scores for each chromosome were usually shared between at least two workers. The figures listed in table III do not include mutations with poor expressivity or those that appeared to pertain to chromosomes other than II. Although few of these visible mutations have been localized to date, we are able to state that none of them showed a tendency to occur repeatedly. Two alleles of *ds* extracted from two different samples differed in their morphological expression and were therefore not of identical origin. Thus, the visible mutations found did not furnish any evidence for consanguinity in the population.

Sex Differences

In each sample investigated some of the chromosomes were derived from wild males, while the remainder stemmed from the sons of wild females. The relative contributions of these two sources to the different viability groups was calculated (table V). As expected no significant difference was found.

Among the unmarked offspring of each test cross, males and females were scored separately. It is well known that in the homozygotes, the deviation from the 50:50% sex-ratio may often be striking, and this fact was confirmed in the present samples. Six chromosomes in all (one in Autumn 1951, two in Spring 1952, and three in Autumn 1952) were characterized by the complete absence of males. All of these chromosomes naturally fell into the lethal or into the deleterious classes. There is no indication that any of these chromosomes were identical, since five of them gave rise to different visible effects in the females. In many other cases, there were fewer females than males. Visible mutations were likewise common in the latter class.

Table V. *Relative contributions of chromosomes originating from wild males and females to different viability groups*

Viability %	♂♂ Flies	♂♂ %	♀♀ Flies	♀♀ %	σ
0– 3.33	239	60.81	154	39.19	±2.46
0–16.66	286	59.83	192	40.17	±2.24
>16.66	456	61.29	288	38.71	±1.79
All classes	742	60.72	480	39.28	±1.40

The sums of males and females among the homozygotes and the sex-ratio for this type were calculated for each sample (table VI). A highly significant excess of females was found in each case. No attempt was made to determine the sex ratio of the *Cy L* type.

Temperature Effect on Viability

During the heat wave in August 1952 we were unable to accommodate all the bottles of the series running, in the available thermorefrigerator. The last trans-

Table VI. *Sex ratio among unmarked flies in five samples from Qiryat 'Anavim*

	No. of unmarked males	%	No. of unmarked females	%	Total unmarked flies
Autumn 1951	12,308	48.89	12,865	51.11	25,173
Spring 1952	18,710	47.89	20,358	52.11	39,068
Summer 1952	17,961	48.12	19.365	51.88	37,326
Autumn 1952	25,957	47.88	28,254	52.12	54,211
Winter 1953	9,651	49.09	10,010	50.91	19,661
Total	84,587	48.21	90,852	51.79	175,439

Chi-square for 1 degree of freedom—223.7

fer of a randomly picked series of 24 chromosomes was therefore left at room temperature and was exposed for five days to a temperature rise of 2.5° C. Only the two bottles kept at 25° C. were considered for classification of the chromosomes involved, while the third bottle was scored separately and was used for an estimate of temperature effects on viability. Although the temperature treatment was brief, four chromosomes were significantly affected. Only one of these shifted decidedly in viability rank. The counts of the first two bottles of this chromosome yielded 32.15% and 34.52% of normal flies, respectively, whereas the third contained only 16.71% (χ^2 with one degree of freedom equals 61.3). Dobzhansky and Spassky (1944) and Dobzhansky (1946) have shown in planned and extensive experiments that the viabilities of certain chromosomes may be modified by temperature to a very considerable extent. The chromosomes utilized by these authors were selected for the purpose of the experiment. Our estimate was made with a randomly picked sample of 24 chromosomes, which turned out to include 8 lethals and 3 semilethals. The result indicates that a short disturbance in temperature will give rise to gross misclassification in only very few (1 ± 1 out of 24) chromosomes.

Allelism Test

While the correlation between lethal content and population size may still be open to discussion, the frequency of allelic lethals could be expected to furnish a more direct indication of the amount of inbreeding prevailing in the population, although even this value might be modified by the adaptive values of certain lethals in the heterozygous condition.

The frequency of allelism was determined for 87 of the lethal chromosomes of the Autumn sample 1952 and for 52 of the Spring sample 1953. All possible crosses were performed for the latter, but not for the former group. Some of the low sublethal chromosomes up to the 3.3% level were included among the lethals utilized. The tests were performed in "creamers" kept at 25° C. and crosses appearing to be allelic were checked in bottles. The test cross was finally accepted as allelic if it yielded 0 or less than 3.3% unmarked flies. The latter case occurred three times. If we had excluded these three crosses from the account we should have been compelled to discard one of the three allelic crosses of the triplet of Autumn 1952. The sublethal chromosomes participated in three allelic pairs, in each instance with a true lethal. In order to avoid any error, which might have been introduced into the experiment by the utilization of the low sublethals, the frequency of allelism was calculated twice (table VII), including or discarding all crosses involving sublethals. The combined rate of allelism of the two samples is not significantly affected by this elimination.

If all the crosses of both samples are considered, the difference in their allelism rate approaches the 0.05% significance level very closely. This difference, at all events, is in the right direction. The incidence of identical lethals should be higher in the spring, if a reduction of the population occurred during the winter. There is not the slightest indication of a corresponding drop in lethal content (table III).

Inversions

No systematic study of chromosome arrangements was attempted. However, for two of the chromosomes extracted from the wild population and subsequently kept balanced with the *Cy L* chromosome the cross-over rate with the latter exceeded by far the expected 1% (cf. Goldschmidt, 1951). Salivary analysis of larvae heterozygous for one of these chromosomes and for the *Cy L* chromosome indicated the presence of an arrangement closely resembling the *In (2L) Cy*. At the time of the investiga-

TABLE VII. *Data on allelism in two samples from Qiryat 'Anavim*

	Sublethals excluded				Sublethals included				Frequency of appearance			
	No. of chromosomes	No. of crosses made	Allelic crosses	Allelism rate	No. of chromosomes	No. of crosses made	Allelic crosses	Allelism rate	1	2	3	4
Autumn 1952	65	1151	5	0.434 ±0.194	87	2092	7	0.335 ±0.126	76	4	1	—
Spring 1953	47	1031	9	0.873 ±0.290	52	1326	11	0.830 ±0.249	38	6	—	1*
Total	112	2182	14	0.642 ±0.171	139	3418	18	0.527 ±0.124	114	10	1	1
Chi-square (1 d.f.)				1.641				3.795				
p				0.3–0.2				0.1–0.05				

* Yielding only 5, instead of 6, allelic crosses. This may be explained by the assumption that two of these chromosomes possess 2 identical lethal loci, a and b, while the third carries only a and the fourth possesses b.

tion, band arrangement appeared completely corresponding in both partners except for about three bands near the chromocentral end of the *Cy* (*2L*) inversion, which, in some heterozygotes, were represented in only one homologue. Thus, we are able to state that *D. melanogaster* in the Judean Hills contains an inversion very nearly homologous with *In* (*2L*) *Cy* and probably identical with the *In* (*2L*) *Cy t* inversion described by Bridges in several laboratory stocks (cf. Bridges and Brehme, 1944) and with the similar inversion identified by Ives (1947) in the population of Massachusetts.

DISCUSSION

The present investigation extending over two years has revealed a more or less uniform concentration of lethal and deleterious second chromosomes in a *D. melanogaster* population inhabiting a fruit-growing district in the Judean Hills in Israel. A sample of second chromosomes from Jerusalem collected during the previous autumn agreed well in its main features with the pooled six samples of the two years' survey. It is a remarkable fact that our values for lethal incidence ($32.16 \pm 1.34\%$) and for lethal and deleterious chromosomes combined ($39.12 \pm 1.40\%$) are paralleled by the data obtained by different authors for several species of the genus Drosophila. The over-all rate for lethals and semilethals found by Pavan *et al.* (1951) in second chromosomes of *D. willistoni* from Brazil amounts to $41.2 \pm 1.1\%$. Dobzhansky and Spassky (1953) found $25.0 \pm 4.0\%$ lethals and semilethals among third chromosomes of *D. pseudoobscura* from California. The corresponding figure for the fourth chromosome is $25.9 \pm 4.2\%$. Since the third and fourth chromosomes of *D. pseudoobscura* are homologous to the right and the left arms of the second chromosome of *D. melanogaster*, we may combine the two figures, correcting for concomitant lethals carried on both arms of the second chromosome of *D. melanogaster* and arrive at a value of $44.4 \pm 5.8\%$, which is not significantly different from the frequency of lethals and semilethals found in our *D. melanogaster* population. *D. persimilis* in California (Dobzhansky and Spassky, 1953) closely resembles *D. pseudoobscura* in lethal incidence. Especial interest attaches to the descriptions of population cages of *D. melanogaster*, kept for numerous generations unirradiated at a large effective breeding size. Levine and Ives (1953) as well as Prout (1954) found that such cages tend to reach an

equilibrium concentration of about 30% of strictly lethal chromosomes. The data for our natural population thus agree with the "optimum" values attained in artificial cage populations.

Whatever significance may attach to this "optimum" level of lethal concentration, it is clear that many natural populations fall short of it. This is true of marginal populations of *D. willistoni* (Townsend, 1952), and of *D. prosaltans* (Dobzhansky and Spassky, 1954) in Brazil. The populations of *D. melanogaster* in Russia (Dubinin, 1946) and in some Northern areas of the United States (Ives, 1954) contain much fewer lethals than found by us in Israel. It is even more difficult, in the light of the knowledge gained from artificial cage populations, to account for those cases, in which the "optimum" level is exceeded. Such an exceptional population was found by Ives (1954) in *D. melanogaster* of Florida, where the concentration of lethals and semilethals amounted to $61.3 \pm 2.3\%$.

The seasonal cycle postulated by Dubinin (1946) could not be confirmed in the present investigation. Although a contraction of the population during the rainy season is indicated by the mean temperature of our collecting area (table I) and by our difficulties and failures to collect flies during the coldest part of the year, no corresponding decrease in lethal content was observed. In this connection it is of interest that Pipkin (1952) found a definite winter contraction of the *D. melanogaster* and *simulans* populations in the neighbouring Lebanon mountains at all six stations visited by her. The reduction in population activity during the cold winter months is very clear cut at Ain Anub (1289 ft above sea level), the locality which appears to be the most comparable in climate to our own collection site.

Systematic records of seasonal variations in lethal concentration are very scarce. Wright, Dobzhansky and Hovanitz (1942) collected *D. pseudoobscura* in California from April 1939 to October 1939 and at one station till March 1940. Their results indicate stability of lethal content throughout the year, although (cf. Pavan *et al.*, 1951) the California populations undergo "sharp seasonal expansions and contractions." Our data point to the conclusion that Mediterranean populations of *D. melanogaster* are comparable to California populations of *D. pseudoobscura* both with regard to the absence of a seasonal "Dubinin cycle" and to the absolute concentration of lethal and semilethal chromosomes.

It is not easy to interpret the data of Dubinin (1946) on *D. melanogaster* as an unequivocal proof of the seasonal cycle postulated by him. These data are compilations of the results obtained by numerous workers and no single investigation at one locality is carried from the autumn of one year to the beginning of the warm season of the next. Thus, we actually lack the figure, which would allow an estimate of the assumed elimination of lethals through inbreeding during the contraction phase. It is also hard to understand the mechanism, which would give rise to the rapid accumulation of lethals during the population peak, such as recorded by some of Dubinin's coworkers. For Uman 1938, Olenov found $21.6 \pm 1.7\%$ lethals in July (21.9% in August (?) according to Dubinin's table VIII, 1946) and $29.8 \pm 1.69\%$ in September. Even when subtracting the standard deviation of each value from this difference, we are left with a gap of 4.8%, which had been bridged during two months at the most, apparently even in one month. Assuming a mutation rate of 0.5% per generation, which is higher than most of the rates recorded by Dubinin, we should need at least nine generations (90 instead of 60–30 days) to effect this change. More probably, a longer period would be required, since the estimates of the mutation rates were based on second chromosomes free of lethals, whereas in Uman 1938 we start off with flies carrying already 20% lethals on their second chro-

mosomes. A second lethal mutation on one of the lethal bearing chromosomes has a chance of 0.1% within each generation, but will not be recorded under the conditions of the experiment. Moreover, back mutations from lethality to normal (Plough, 1942) should also occur, counteracting to an unknown extent the mutation pressure directed toward lethality. Finally, in consideration of the high rate of allelism among lethals of the Russian populations, a certain amount of elimination through homozygosis should occur even during the expansion phase. Clearly, the mutation rates recorded by Dubinin do not suffice to account for a "Dubinin cycle" of the postulated amplitude. In the experiments of Levine and Ives (1953) 84 days were required to raise the lethal incidence in a population cage at 25° C. from 1.3 ± 0.75% to 4.3 ± 1.6%. Dubinin himself, while assuming that the lethal concentration might actually rise by 12 or possibly by 26% during one season, remarks that this accumulation would be too rapid to be accounted for solely by mutation pressure. If, however, the mutation rate is as low as found in the experiments of Levine and Ives (1953) and if mutation pressure alone should be responsible for some increase of lethals during the summer, we should have had to work with samples five- to tenfold as large as our present ones in order to verify the resulting fluctuations with statistical significance.

Pooling the first, second and last samples, respectively, collected during several years in Amherst, Massachusetts, Ives (1954) noted an almost significant tendency for increase in lethal content with the advance of the season. However, "the increase is not as large as the average increase in Russian populations." On the other hand, Ives' (1954) data reveal highly significant "long range" changes in the Massachusetts as well as in the Florida populations, consisting in a reduction of lethal content during a period of more than ten years.

Our results indicate a barely significant difference between the concentrations of allelic lethals in the Autumn 1952 and in the following Spring. If allelism is inversely related to population size, we might interpret this difference as a corroboration of the contraction during the cold season, which we have to postulate for climatic reasons. The data of Wright *et al.* (1942) as well as those of Ives (1954) for Massachusetts (but not for Florida) contain indications that allelism is indeed negatively correlated with the effective size of the breeding population. For this reason it is important to stress that at our locality the apparent increase

TABLE VIII. *Lethal and allelism rates for second chromosomes in different populations of* Drosophila melanogaster

Authors	Locality	Date	Lethal incidence %	Lethal and semilethal incidence %	Allelism incidence %	Repeated chromosomes
Dubinin, 1946	Gelendzhik	July 1933	7.9±0.9			29 in 49
Dubinin, 1946	Gelendzhik	July 1934	12.6±1.3			33 in 88
Dubinin, 1946	Simferopol	Oct. 1939	24.1±1.6			48 in 128
Dubinin, 1946	Kutaisi	Nov. 1939	38.9±2.2			44 in 88
Ives, 1954	Mass.	1938–1946		48.8±2.2	0.39±0.130	
Ives, 1954	Mass.	1947–1952		35.9±1.4	0.92±0.121	
Ives, 1954	Florida	1940, 1942		65.3±2.6	0.44±0.139	
Ives, 1954	Florida	1951		51.1±4.4	0.38±0.169	
Present	Q. 'Anavim	Oct. 1952	33.6±2.8	42.3±2.9	0.34±0.126	
Present	Q. 'Anavim	May 1953	34.8±3.1	42.0±3.2	0.83±0.249	16 in 52
Present	Q. 'Anavim	All data	32.2±1.3	39.1±1.4	0.53±0.124	
Prout, 1954	Population cage (3) (unirradiated)		about 30%		0.52±0.297	6 in 65

in allelism after the Winter 1953 was not accompanied with a reduction in lethal incidence. Levine and Ives (1953) found that the frequency of lethals in a population cage dropped from the initial 37.9% present in the wild population to 22.9% as a first result of the inbreeding resulting from the stocking of the cage with a small number of flies. Thereafter, lethal frequency rose once again, fluctuating around a value of $29.24 \pm 2.01\%$, while allelism in this cage was higher than in the initial sample of the wild population. This fact would tend to confirm our own impression that a population may preserve a fairly constant concentration of lethals around 30%, while both its breeding size and its allelism undergo fluctuations.

Table VIII is a compilation of some results obtained by different workers on allelism and lethal concentration. Dubinin was dealing with a lower incidence of lethals than found in Israel, and, at the same time, he obtained much higher rates of allelism. However, allelic rate was very high even in the Kutaisi population of 1939, the only one exceeding that of Qiryat 'Anavim in lethal content. Allelism in Massachusetts (Ives, 1954) during 1938–1946 was close to our autumn value, while since 1947 it has approached our estimate for the spring sample. The agreement existing between our own population and an unirradiated cage population of "infinite" size described by Prout (1954) can hardly be ascribed to chance. While Ives (1954) justly stresses "that in a natural population the combination of important variables and effective agents is probably so complex that only very general applications can be made of the findings from studies of artificial cage populations," this perfect match between a natural and an artificial population appears the more remarkable.

The theory of the dynamics of lethal genes in populations is mainly based on their effect in the homozygote. The existing knowledge on viabilities of lethal heterozygotes (Stern *et al.*, 1952; Cordeiro, 1952) has never been adequately incorporated into the argumentation, although as early as 1942 the importance of this aspect was stressed by Wright *et al.* The results of Dubinin (1946), Ives (1945, 1954) Levine and Ives (1953) as well as our own include data which are hard to account for on the simple assumption that the accumulation of lethals is solely due to mutation pressure and that their elimination is effected exclusively in the homozygous state. The incidence of lethal genes and their allelism is not in all cases inversely related. Under these circumstances we hesitate to rely on the classical theory and to interpret the discrepancies in lethal frequency between various American, Russian and Israel populations of *D. melanogaster* as an unequivocal proof of corresponding differences in the effective sizes of the breeding populations of the fly in the areas concerned.

Summary

1. The viabilities of homozygotes for second chromosomes extracted from a wild population of *D. melanogaster* in one locality in Israel were investigated in six consecutive samples collected during two years.

2. The incidence of lethal chromosomes in these six samples fluctuated about a value of $32.16 \pm 1.34\%$ and the concentration of lethal and semilethal chromosomes combined amounted to $39.12 \pm 1.40\%$.

3. The six samples did not differ significantly among one another in the sizes of the various viability classes. Thus, although the collection data indicate a population bottle neck occurring during the rainy winter months, a corresponding reduction in the concentration of lethal genes, as recorded by Dubinin for Russian populations of the fly, could not be observed.

4. The concentration of deleterious recessive genes in the present six samples showed good agreement with the data of

a previously investigated population from Jerusalem.

5. Among the visible mutations extracted from the 1,222 chromosomes of the present samples no evidence of duplicity due to inbreeding was discovered.

6. The average viability of the "normal" homozygote appeared to be consistently larger (30.20%) in the population from the Judean Hills than in the sample from Jerusalem (27.98%).

7. There was a highly significant excess of females among the unmarked flies recovered in the test generation.

8. An inversion very nearly homologous with *In (2L) Cy* was found in the present population.

9. The frequency of allelism amounted to $0.335 \pm 0.126\%$ among the lethals of the autumn sample of 1952 and to $0.830 \pm 0.249\%$ in the sample of the following spring. This difference very nearly reaches the 0.05% probability level and might therefore be interpreted as an indication of a reduction in the effective size of the breeding population during the rainy season.

10. It is shown that some of the data obtained by various investigators do not substantiate an inverse relationship between lethal concentration and allelism.

11. It is suggested that lethals on the second chromosome of *D. melanogaster* and on its homologues in some other Drosophila species frequently tend to fluctuate around an optimum concentration near 30 percent.

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THE ADAPTIVE AND PHYLOGENETIC SIGNIFICANCE OF VERTEBRAL FORM IN SNAKES [1]

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INTRODUCTION

Studies of the origin and subsequent evolution of the snakes are severely limited by the nature of the fossil remains of this group. As the fragile skull is seldom recovered, knowledge of the history of snakes must be gleaned from the infrequent finds of scattered vertebrae and from speculations upon the modern fauna. The necessary concentration upon vertebral anatomy in the study of the record has resulted in an imposing vocabulary, but has yet to reveal any information that may be usefully applied to an understanding of the relationships between the various sub-groups as they appear today. Examination of the modern fauna has not fully clarified the relationships of major groups since evidence from the skull, musculature and scalation has given rise to conflicting opinions dependent upon the kind of morphological data studied.

These difficulties are most certainly the consequence of studying an animal whose high degree of specialization obscures an underlying continuity. To unravel phylogeny from last minute specialization, we seek stable characters. The relative stability of the vertebral column in the history of other groups recommends this system for such a study. Further, a review of the vertebral characteristics of modern snakes is primary to an interpretation of their record, such as it is.

The present study is a partial analysis of the variation in the form of the vertebrae of snakes and an attempt to associate particular patterns of variation with mode of life and phylogenetic lines. The distinctive features of particular snake vertebrae are not immediately obvious and the subtleties of adaptation must be sought with sensitive methods. Therefore an attempt has been made here to develop a statistical program capable of revealing the biological significance of an abstruse morphology. Such a program must be capable of treating super-specific groupings, and so its operations must be independent of the severely limiting assumptions of the standard statistical tests.

Since an unwieldy number of characters could be considered, it is first necessary to choose those measures that may be expected to economically represent a vertebra in the context of this study. The measures employed must also be of use in the practical application of the conclusions drawn here. Therefore, the first part of this work is devoted to a search for characters in accordance with several such criteria. The suite obtained will be applied to the problem of ascertaining the extent to which the vertebrae of snakes are subject to specialization. With knowledge of the gross adaptive significance of vertebral form, it will then become possible to examine the array for their phylogenetic implications.

MATERIALS

The majority of specimens used in this study were obtained on loan from the excellent collections of the Chicago Natural History Museum (C.N.H.M.). Australian materials were supplied by Mr. Wendell Swanson (W.S.), and the remainder from a small personal collection (R.G.J.). The following snakes were used in the analysis:

[1] Offered in partial fulfilment of the requirements for the degree of Doctor of Philosophy to the faculty of the Division of Biological Science, The University of Chicago, June 1954.

Anilidae
Cylindrophis rufus CNHM 69980
Boidae
Boinae
Boa canina CNHM 27018 arboreal
Charina bottae CNHM 22348 fossorial
Constrictor constrictor constrictor CNHM 22360 arboreal
Enygrus carinatus CNHM 44268 arboreal
Eryx conicus CNHM 48828 fossorial
Eryx johnii CNHM 31319 fossorial
Eunectes murinus CNHM 22776 aquatic
Lichanura roseofusca CNHM 31342 terrestrial
Pythoninae
Chondropython viridis CNHM 14075 arboreal
Morelia argus variegatus WS arboreal
Achrochordidae
Chersydrus granulatus CNHM 53559 aquatic
Colubridae
Boiginae
Boiga irregularis WS 17 arboreal
Imantodes cenchoa leucomelas CNHM 70747 arboreal
Oxybelis fulgidus CNHM 22376 arboreal
Passerita prasinus CNHM 71633 arboreal
Colubrinae
Abastor erythrogrammus CNHM 38114 fossorial
Atractus nicefori CNHM 54881 fossorial
Coluber taeniatus ruthveni RGJ terrestrial
Dendrophis punctulatas WS 18 arboreal
Geophis chalybaeus CNHM 20354 fossorial
Lampropeltis getulus holbrooki RGJ 50 terrestrial
Liodytes alleni CNHM 25944 aquatic
Natrix erythrogaster transversus CNHM 46325 aquatic
Natrix rhombifera CNHM 46307 aquatic
Natrix sipedon confluens CNHM 46300 aquatic
Natrix tasselatus CNHM 20893 aquatic
Opisthotropis kuatunensis CNHM 24869 aquatic
Thamnophis eques RGJ 15 terrestrial
Thamnophis sauritus proximus RGJ terrestrial
Homalopsinae
Cerberus rhynchops CNHM 53298 aquatic
Enhydris doriae CNHM 14919 aquatic
Fordonia leucobalia CNHM 71592 aquatic
Homalopsis buccata CNHM 11551 aquatic
Elapidae
Acanthophis antarcticus WS terrestrial
Dendraspis viridis CNHM 22595 arboreal
Doliophis philippinus CNHM 68918 fossorial
Naja naja naja CNHM 26842 terrestrial
Notechis scutatus RGJ 51 terrestrial
Pseudechis porphyriacus RGJ 52 terrestrial
Walterinnesia aegyptia CNHM 72321 terrestrial
Hydrophidae
Hydrophinae
Hydrophis belcheri CNHM 13611 aquatic
Pelamis platurus CNHM 41590 and 9774 aquatic
Laticaudinae
Laticauda colubrina CNHM 13938 aquatic
Crotalidae
Ancistrodon contortrix RGJ 18 terrestrial
Ancistrodon piscivorus CNHM 37547 aquatic
Crotalus atrox RGJ 12 and 19 terrestrial
Crotalus cerastes CNHM 26163 terrestrial

Crotalus horridus CNHM 45092 terrestrial
Trimeresurus schlegelii CNHM 2524 arboreal
Trimeresurus wagleri CNHM 71624 arboreal

Viperidae

Atheris nitschii CNHM 8987 arboreal
Atractaspis microlepidota CNHM 62104 burrower
Bitis gabonica CNHM 22254 terrestrial
Cerastes cerastes CNHM 69252 terrestrial

A deliberate effort was made to make this sample as heterogeneous as possible with respect to both taxonomy and habitus. Eight of the 12 extant families are included in the sample of 57 specimens. The predominance of the Colubridae (22) is in accord with the eminence of this group in the modern world fauna. Notable omissions are evident, such as the interesting families Typhlopidae and Leptotyphlopidae. Although the use of x-rays in this study made available many more specimens than could have been obtained from the local osteological collections, the size or state of preservation of these and other desirable snakes prevented their use.

No attempt will be made to justify the mode-of-life classifications beyond an example of such a judgment. The habits of the rare cobra *Walterinnesia aegyptia* are unknown, but because the few specimens known are from well populated areas it has been assumed to be secretive or even burrowing. While a few anatomical features such as the rounded snout and slightly reduced eyes suggest specialization towards the burrowing habit, *in toto* the animal does not have the appearance of a confirmed burrower. In the absence of habit records, the snake has been classified as terrestrial. Similarly for each snake, references were consulted for habits and habitat preference and the general external appearance considered in the assignment.

These categories are admittedly gross, and it is recognized that many quite divergent modes of life have been lumped within a single grouping. To anticipate the conclusions of this study, this bias does not appear to be of significance. Seventeen terrestrial, 17 aquatic, 14 arboreal and 9 burrowing snakes are included in this sample.

Techniques

The data used in the analysis were obtained from x-rays. The procedure and a consideration of the factors of distortion have been described elsewhere (R. G. Johnson, 1955). The advantages of x-rays in studies such as this lie in the speed of the method, and the greater availability of specimens. The drawbacks arise from the distortion inherent in the nature of the x-ray projection.

The degree of distortion under the conditions of the physical set-up used, and the positioning procedure employed, was assessed in three ways. The theoretical magnifications for the source-film distance and the range of object-film distances encountered were calculated for the conditions of the greatest expected error. These were found to range from 1 to 3 per cent. Calipers open to a known distance were x-rayed at various heights above the film. The calipers were placed so as to be as far from the central ray as any structure x-rayed in this study. The radiographic image of the calipers was measured and the results were found to agree with the theoretical expectations. The conclusive assessment was made by recording a specimen, cleaning or clearing and then measuring the actual object of study. A range of 1.5 to 3 per cent distortion was observed in tests of the material used in this study.

The two sources of distortion of a radiographic image, projection and positioning, affect image size in opposite ways. The x-ray projection tends to magnify and the effect of tipping the object tends to reduce. Therefore, to a limited extent the

tendencies cancel each other. The combined effect of these factors is further reduced by the use of ratios between measures on the same structure. If two measures obtained from the same exposure, and made along the same axis are combined, the factors of distortion can be regarded as affecting each equally. The value of the ratio was assumed to remain the same regardless of the degree of distortion. The assumption is justified only on small structures, as encountered in these materials, where the distances between the measures entering the ratio are quite small. The repeatability of measurement was found to be within 1 to 2 per cent error, so that in view of the assessments of distortion and the above considerations, error resulting from the use of x-rays was disregarded.

Measurements were made on a special light box equipped with a cross-hair scanner travelling on a vernier scale reading to tenths of a millimeter. Ten vertebrae from each snake were measured. A consideration of this sampling will be found in the discussion of the choice of measures. About 4,000 separate measurements entered into the analysis, while twice this number were made in the course of the preliminary work.

The Choice of Characters

It is desirable that the characters chosen for this study have certain attributes. Needless to say, they must be readily available from radiographs. They should have a biological meaning in the sense that they adequately represent features that may be translated into functional requirements.

The character employed must be independent of absolute size, in view of the effect of environment and nutrition on growth, and the fact that reptiles continue growth throughout life. The use of ratios appears to be a satisfactory way of circumventing the effect of these factors. While relative growth might be expected to alter the value of a ratio during life, the striking coherency of individuals of the same or closely related species with respect to the ratios investigated, suggests that this variable may be neglected. Furthermore, the consequences of relative growth need not be considered in the inquiry into the adaptive significance of vertebral form as pursued in this study. Irrespective of change in proportion during life, the animal is involved in a particular set of activities when abruptly it enters the sample. It is the association of these activities and the vertebral morphology functioning under the requirements of these activities, that is of central concern. The characters to be studied, then, will be ratios of two measures made upon the same vertebra.

It is also desirable that the characters studied show relatively low intra-columnar variances. There are two practical reasons for this criterion. First, it is hoped that the conclusions of this work will eventually be applied to the problem of interpreting the fossil record of snakes. Since no satisfactory method for determining the column position of isolated vertebrae has as yet been devised, we must seek characters that are least affected by position. The vertebral column of snakes may be divided into cervical, thoracic and sacral regions. Further division of the thoracic into anterior thoracic and posterior thoracic sub-regions is possible on the basis of micro-structures and the occurrences of hypapophysis (Sood, 1948; Gilmore, 1938). There is no clear boundary between these subdivisions. The atlas and axis are apparent and the caudal vertebrae clearly distinguishable, but the greater part of the column is for practical purposes a spectrum. I found, by measuring every vertebra on a cleaned skeleton of *Constrictor constrictor imperator,* that differences between adjacent vertebrae were often imperceptible and seldom exceeded a tenth of a millimeter.

There is a more fundamental argument for considering only characters exhibiting low intra-columnar variance concerned with the necessity of representing a character of a metameric organ by a single value. The comparison of the entire suite

of values a given ratio may take, throughout a column between columns, would pose a major statistical problem. To compare individual, analogous vertebrae, requires us to establish their identity from snake to snake. This difficulty may readily be appreciated by considering the task of this identification on a colubrid with 120 thoracic vertebrae and a boid with 330 thoracic vertebrae. If, however, only ratios with characteristically low intra-columnar variance are employed, it is practical to represent a given character by the mean or median of its values throughout a column.

A preliminary examination of the data suggested that the mean satisfactorily represents a character's expression in the column. In the analysis of clusters of such means, with reference to mode-of-life or taxonomic categories, it would be convenient to neglect the intra-columnar variance of the means representing individual columns. This is justified in a rigorous sense only when these variances are not significantly different. The practical necessity of treating materials that do not behave in a manner as statistically satisfying as physical phenomena, forces a less rigorous justification. The neglect of intra-columnar variance is rationalized as permissible if the ratios used exhibit relatively low intra-columnar variance and on the general observation that the frequency distributions of the intra-columnar values are similar in kind for all snakes for a particular ratio.

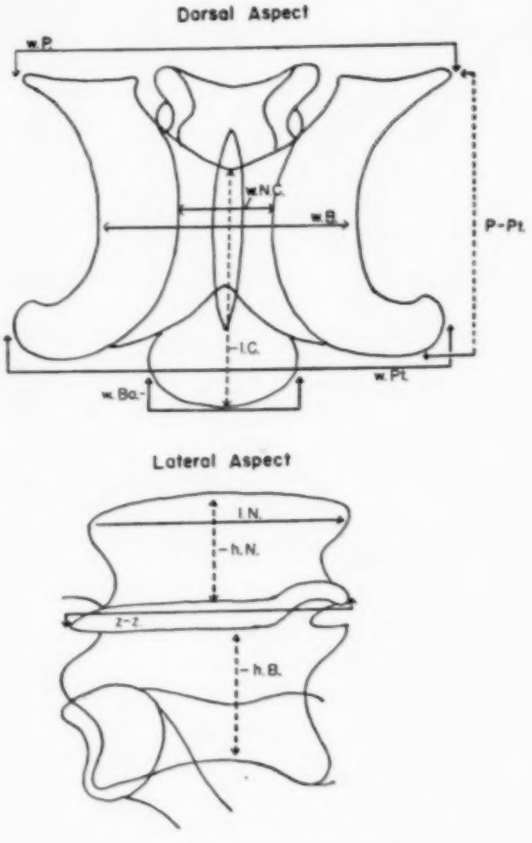

FIG. 1. The measures.

Choice of measures. Only thoracic vertebrae were considered in this study. Caudal vertebrae are quite complex and appear to be highly variable. As the thoracic vertebrae outnumber the caudals, they are more commonly encountered in the geological record.

Twelve measures were found to be readily available from the radiographs and also judged biologically reasonable. They are, as illustrated in figure 1:

Dorsal Aspect:

- w.P.—greatest width across the prezygapophyses
- w.Pt.—greatest width across the postzygapophyses
- w.B.—least width of body
- w.NC.—least width of the neural canal
- w.Ba.—greatest width of the condyle
- P-Pt.—greatest distances between the pre- and postzygapophyses
- l.Ba.—greatest length of the condyle
- l.C.—length of the centrum along the midline, from the deepest point within the glenoid cavity to the posterior edge of the condyle.

Lateral Aspect:

- h.B.—least height of body from the ventral edge of the centrum to the zygantrum-zygosphene ridge
- h.N.—greatest height of the neural spine above the zygantrum-zygosphene ridge
- z-z.—length of the zygantrum-zygosphene ridge
- l.N.—greatest distance between the anterior and posterior edges of the neural spine.

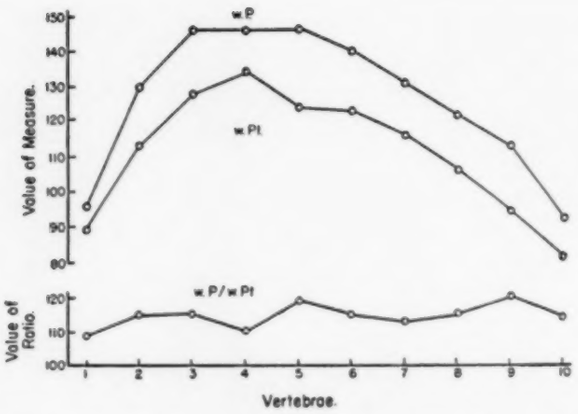

FIG. 2. Comparison of columnar variability of two measures and their ratio (*Naja*).

Since it is not economical to measure every vertebra in a column, it is necessary to select a sample that will efficiently represent the values assumed by a particular measure, consistently from snake to snake. The variation of a particular measure down the column is regular, so that it is possible to sample each column systematically. As there is a greater chance of misrepresenting such a small population of this nature by random sampling, systematic sampling of a column will tend to be less biased. The utilization of ratios with low intra-columnar variance, in addition, will be expected to diminish the magnitude of any sampling prejudice (fig. 2).

An examination of measures made upon each vertebra of several snakes indicated

TABLE 1. *The choice of characters. All possible ratios and the qualitative selection made among them. Combinations marked ⁻ were chosen for further study*

	Dorsal aspect							Lateral aspect			
	w.Pt.	w.B.	w.NC.	w.Ba.	P-Pt.	l.Ba.	l.C.	h.B.	h.N.	z-z.	l.N.
w.P.	0	0	0	0	–		–				
	–	–	–	–	–		–				
w.Pt.		0	0	0	–		–				
		–	–	–	–		–				
w.B.			0	0	–		–	–			
			–	–	–		–	–			
w.NC.				0			–				
				–			–				
w.Ba.						–					
						–					
P-Pt.						0	0				
						–	–				
l.Ba.							0				
							–				
l.C.								–	–	–	–
								–	–	–	–
h.B.									0	–	–
									–	–	–
h.N.											–
											–
z-z.											0
											–
l.N.											

Minimum radiographic distortion—0.
Biologically reasonably (as defined in text)—⁻.

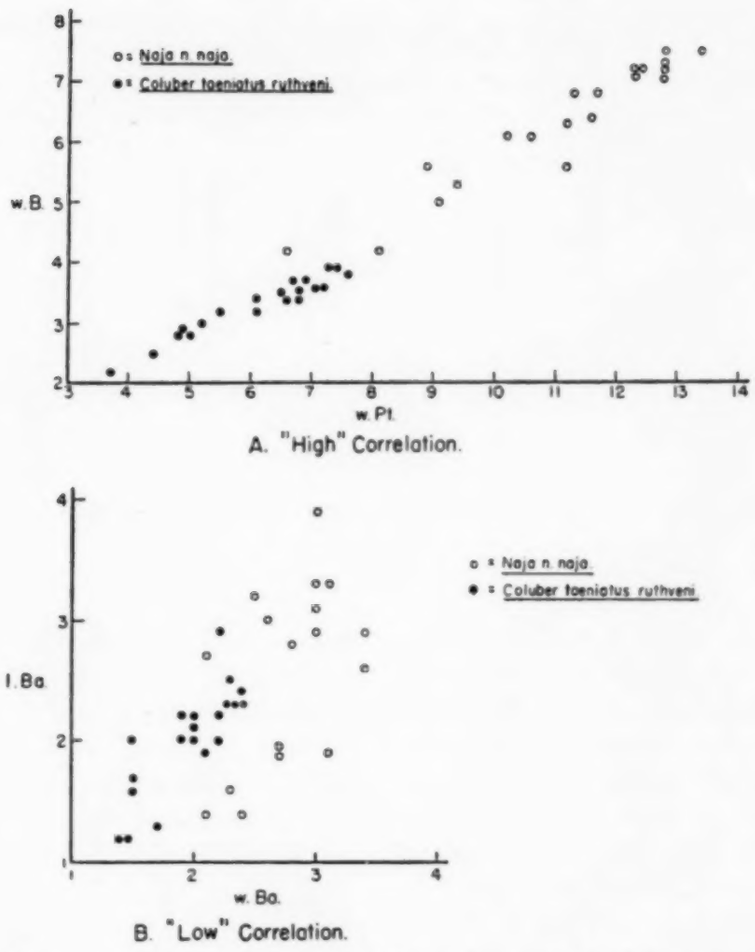

FIG. 3. Sample scatter diagrams illustrating the judgments of high and low correlation (20 vertebrae/snake).

that a sample of ten vertebrae taken equal distances apart would adequately represent the column in a manner consistent between snakes. The first vertebra measured was always the fifth, for it is usually one of the first in the column to assume a typical thoracic appearance.

Choice of ratios. All possible combinations of the twelve measures selected yield sixty-six ratios. Many of these ratios, however, express the same relationships in a variety of ways. Others express a vertebral feature that is not an obvious characteristic of general configuration, or that cannot be interpreted within our limited knowledge of function. Finally, still others associate measures made from radiographs of the dorsal and lateral aspects and thereby introduce an additional error. In view of these objections, only thirty-one of the total possible ratios were selected for further study as shown in table 1. In several instances radiographs were crossed where the ratios were of particular interest, but as could be expected, these did not survive the analysis of intra-columnar variability to follow.

Pairs of measures showing high correlation down the column would be expected to yield ratios of low intra-columnar variance. Therefore scatter diagrams were plotted for each of the thirty-one ratios for each of 5 snakes. The sample represented all 4 habitus groups and 4 of the families. The ratios were sorted into "high" and "low" correlation categories. All diagrams were compared and a qualitative judgment upon the extent of the scattering of the points was made on each. Whenever difficulty was encountered in deciding to which class a particular scatter diagram belonged, the plot in question

TABLE 2. *Intracolumnar variability. The qualitative judgments made upon the scatter diagrams of these measures in* Naja *and* Coluber *(20 vertebrae/snake)*

	w.Pt.	w.B.	w.NC.	w.Ba.	P-Pt.	l.Ba.	l.C.	h.B.	h.N.	z-z.	l.N.
w.P.	x*	x	x	x	x		x				
w.Pt.		x	x	x	x		x				
w.B.			x	x	x		x	o*			
w.NC.				x			x				
w.Ba.						o					
P-Pt.						o	x				
l.Ba.							o				
l.C.								x	o	o	x
h.B.									x	o	o
h.N.											o
z-z.											x

* Key: x—"high" correlation.
o—"Low" correlation.

was placed in the "high" correlation class. Representative judgments are shown in figure 3. Twenty-two of the 31 ratios survived (table 2). Since all 5 individuals in this heterogeneous sample placed as a group in either the "high" or "low" category for each ratio judged, the sample of 5 snakes was accepted as adequate.

The variance of each of these survivors was calculated from a sample of 10 vertebrae from each of 10 snakes. The sample represented all 4 mode-of-life categories and 6 of the families. The intracolumnar variability of these ratios, expressed as coefficients of variability (C.V. $= 100 \times$ standard deviation/mean), are shown in table 3. On examination of these coefficients, 12 of the 22 ratios were selected as showing, more or less consistently, low variability throughout the sample. None of the ratios made upon the lateral aspect of the vertebra survived and so these measures were discarded. The judgment was necessarily arbitrary, since there is no convenient reference to which these values may be compared, except the array itself. To limit the choice to ratios showing no significant differences between coefficients of variability from snake to snake, would eliminate all but a very few of the ratios.

In summary, the character to be studied will be taken as an average ratio of 2 measures from each of 10 vertebrae for every snake. Of the 66 ratios possible between the 12 measures chosen, 12 were selected as being biologically meaningful and exhibiting relatively low intra-columnar variance. These ratios are:

1. w.P./W.Pt.
2. w.P./w.B.
3. w.P./P-Pt.
4. w.Pt./w.B.
5. w.Pt./w.Ba.
6. w.Pt./P-Pt.
7. P-Pt./1.C.
8. 1.C./w.B.
9. w.B./w.NC.
10. w.P./1.C.
11. w.Pt./1.C.
12. P-Pt./w.B.

ANALYSIS

The purpose of the analysis is to determine the adaptive and phylogenetic significance of vertebral form as represented by the suite of characters chosen for study. The analytical model is organized as follows:

I. Analysis of the classification by mode-of-life.
   A. Consider vertebral characters 1 to 12 and modes-of-life terrestrial, aquatic, arboreal and fossorial. To distinguish terrestrial from aquatic, etc. by the quantitative expression of characters 1 to 12.
   B. Assume each mode-of-life group to be as taxonomically heterogeneous as any other mode-of-life category.

TABLE 3. *Intracolumnar variability. Coefficients of variability (V) for a sample of ten snakes (10 vertebrae/snake)*

Character	*Coluber*	*Crotalus horridus*	*Hydrophis*	*Natrix Sipedon Confluens*	*Thamnophis Saurilus*	*Naja*	*Crotalus Atrox*	*Atractaspis*	*Dendraspis*	*Eryx Conicus*	Acceptable*
w.P./w.Pt.	9.8	8.9	9.6	5.6	5.6	2.9	5.6	3.7	4.0	5.0	*
w.P./w.B.	11.1	11.8	15.1	9.3	5.4	6.7	8.4	7.4	9.7	9.1	*
w.P./P-Pt.	8.8	8.3	7.1	9.0	5.6	4.5	7.4	6.2	6.1	7.5	*
w.Pt./w.B.	5.5	6.6	7.6	3.9	11.1	5.8	6.2	5.7	8.1	8.3	*
w.Pt./w.Ba.	8.7	13.5	11.0	11.3	13.9	9.3	8.5	9.7	11.7	11.3	*
w.Pt./P-Pt.	7.5	8.8	15.3	6.5	6.3	5.1	6.5	7.7	4.8	6.5	*
w.B./w.Ba.	8.2	18.7	15.1	8.7	20.8	6.9	10.3	13.0	11.1	10.7	
w.Ba./w.NC.	8.0	7.7	12.6	9.4	36.1	8.7	16.7	9.1	16.8	14.6	
P-Pt./l.C.	5.6	3.0	7.5	5.4	6.6	4.8	5.4	11.8	3.9	4.4	*
l.C./w.B.	10.6	11.7	15.1	7.6	5.4	10.2	13.7	12.4	10.5	13.4	*
w.P./w.NC.	15.3	18.7	15.8								
w.P./w.Ba.	12.9	14.8	13.8								
w.Pt./w.NC.	13.8	14.1	6.7	15.2							
w.B./w.NC.	6.8	11.3	7.2	5.8	20.5	17.0	14.7	10.1	9.2	14.6	*
l.C./w.NC.	6.7	14.4	13.4	9.0	16.2	15.2	10.9	13.2	10.2	15.1	
l.C./l.N.	7.9	18.3	45.7								
z-z./l.N.	8.9	10.3	37.5								
w.B./h.B.	16.6	15.8	11.8								
l.C./h.B.	5.4	25.5	17.5								
h.B./h.N.	11.0	24.6	29.2								
w.P./l.C.	11.6	11.3	7.6	9.7	10.8	6.6	9.2	11.3	8.0	10.2	*
w.Pt./l.C.	7.2	13.1	7.9	8.7	8.9	7.9	8.7	10.6	6.3	8.2	*
P-Pt./w.B.	5.3	10.9	18.9	9.5	8.7	6.7	11.8	10.6	7.3	11.9	*

C. For each character, test the hypothesis that the mode-of-life samples were drawn from the same population.

II. Analysis of the classification by families.

A. Consider vertebral characters 1 to 12 and families Boidae, Colubridae, Elapidae, etc. To distinguish between families by the quantitative expression of characters 1 to 12.

B. Assume each taxonomic category as to be heterogeneous with respect to mode-of-life as any other taxonomic group.

C. For each character, test the hypothesis that the taxonomic samples were drawn from the same population.

The inferences to be drawn from this model are to the universe of all snakes of these modes of living and of these families. The elements of this universe are individual vertebral columns. To each of these elements is attached a measure, the character as defined previously. The model essentially tests the hypothesis that this universe may be subdivided into discrete ecological and/or taxonomic categories by each of the characters studied.

Two assumptions were made in the model, equal taxonomic heterogeneity in the mode-of-life classes and equal mode-of-life heterogeneity in the taxonomic categories. The latter seems justifiable, the former less so. In one instance, that of the comparison of terrestrials against aquatics, the preponderance of colubrids in the aquatic sample was unavoidable. Additional tests were necessary to discover the effect of this family.

The gross nature of the mode-of-life classification has been previously noted. Within each of these categories more spe-

cific adaptive types may be recognized as follows:

Terrestrial
- General terrestrial (*Pituophis, Thamnophis*)
- Swift terrestrial (*Coluber*)
- Secretive (*Storeria*)
- Desert terrestrial-sidewinders (in part *Crotalus, Cerastes*)

Aquatic
- General aquatic (*Cerebrus*)
- Riparian (*Thamnophis* in part)
- Marine (*Hydrophis*)

Arboreal
- Arboreal I.—vine snake forms (*Boiga, Dendrophis*)
- Arboreal II.—thick bodied forms (*Trimeresurus* in part, *Boa*)
- Semi-arboreal (*Morelia*)

Fossorial
- Subterranean (*Typhlops*)
- Mud burrower (*Xenopeltis*)
- Leaf mold burrower (*Anilius*)
- Sand burrower (*Eryx*)
- Semi-fossorial (*Micrurus* in part)

If this classification were to be used in its entirety, a sample of at least twice the number in the present sample would be required. It would be quite difficult to find more than several representatives of many of the classes. In the writer's opinion, the gain in information would not be in proportion to the additional effort necessary.

The standard statistical procedures, such as the analysis of variance and the t-test, could not be justifiably applied to these data for they assume conditions not met in such a genetically heterogeneous population. There is no *a priori* reason to expect that the characters under consideration are normally distributed within the universe or the subdivisions of the universe sampled. Nor can the assumption of homogeneous variance within these categories be admitted. Indeed, the sample would indicate otherwise.

In view of these restrictions, nonparametric statistics, resting upon only very general assumptions, have been employed. Three tests have been applied to 2 forms of the hypothesis that the subsamples are not different; the Kolmogorov-Smirnoff statistic, the H-test of Kruskal and Wallis and a rank test for small samples designed by Colin White.

The Kolmogorov-Smirnoff statistic can be applied to test the hypothesis that two sample frequency distributions are drawn from the same population. A step-cumulative frequency curve is plotted for each of the samples, the y-axis expressed in per cent of each. The percentage of maximum vertical difference between two such curves is solved graphically. The critical value for maximum vertical difference is obtained from the statistic

$$x\sqrt{(1/n_1) + (1/n_2)}$$

where n_1 and n_2 are the respective sample sizes and x is a constant for a particular level of significance [2] (Dixon and Massey, 1951, p. 257).

The advantages of this test are several. As far as is known, there are no restrictive assumptions about population distribution inherent in it. Furthermore, it is a comprehensive test in that it compares the samples *in toto*. In a test of the hypothesis that two samples were drawn from different populations, a significance difference between parameters is not necessarily pertinent if the populations differ in species of frequency distribution. The test is rapid once the scales have been adjusted, since many samples may be compared on the same graph. Samples of as few as 3 or 4 observations can be accommodated.

There is one drawback to the test as used here. Significant differences between two samples are found in terms of their frequency distributions. The test can show that two samples are drawn from different populations, but the difference is not in the terms most frequently employed in biological interpretation.

[2] The writer is obliged to Dr. Robert Lee Miller for suggesting this test.

Therefore, once the difference between two samples had been accepted on the basis of this test, the difference in central tendency was determined. With the knowledge that two samples were different, it was desirable to inquire into the nature of these differences.

The H-test of Kruskal and Wallis was used to test the difference in central tendency. It is not known whether or not this rank test is absolutely equivalent to a comparison of means. In the words of its designers, ". . . what H really tests is a tendency for observations in at least one of the populations to be larger (or smaller) than all observations together when paired randomly" (Kruskal and Wallis, 1952). They point out that it is the consensus of statistical thinking that in practice, however, a rank test such as the statistic H is fairly oblivious to differences in variability and that it may be employed in comparing means without the assumption of equality of variances. This consideration as well as the general statistical efficiency of rank tests has not yet been resolved mathematically.

The statistic H is distributed as X^2 $(C-1)$, where C is the number of samples, when the n_i are greater than 5. When at least one of the samples contain five or less observations a rank test by White has been used (White, 1952).

TABLE 4. *Kolmogorov-Smirnoff test. Per cent differences for the characters showing significant difference ($P=0.05$) in the mode of life samples and the critical level for the rejection of the null hypothesis*

	Sample	Critical K-S.
Terrestrial vs. Aquatic		
w.P./w.Pt.	54.2%	45.9%
w.Pt./w.Ba.	53.9%	45.9%
Terrestrial vs. Arboreal		
w.P./P-Pt.	50.6%	49.5%
Terrestrial vs. Fossorial		
w.Pt./w.B.	67.0%	55.5%
Aquatic vs. Arboreal		
w.P./w.Pt.	80.0%	50.1%
Arboreal vs. Fossorial		
w.P./P-Pt.	61.7%	56.1%

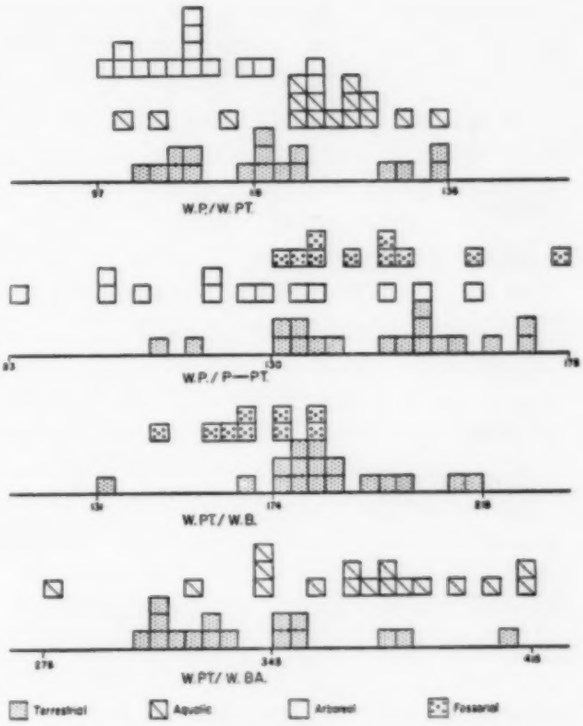

FIG. 4. Frequency diagrams of mode of life categories showing significant differences.

Tables for White's test are available for the conditions of small sample size, when the sum of all observations is equal to or less than 30 and when only two samples are being considered.

The rank tests involve only broad assumptions. They assume that the observations are all independent, that a given sample is from a single population and that the distributions of the samples being compared are roughly of the same form (Kruskal and Wallis, 1952). These restrictions appear to be admissible here.

ANALYSIS OF MODE OF LIFE

The Kolmogorov-Smirnoff test was performed for each character between each mode-of-life class. The degree of specialization so revealed confirms the thesis of the relative stability of the vertebral column in snakes. Of the seventy-two comparisons made, only six instances were found in which a pair of classes differed significantly. The characters involved in these cases are shown in table 4, and their distribution in the samples exhibited in figure 4. In the discussion of these results

TABLE 5. *Summary of results of White's test on Colubridae of various modes of life hypothesis rejected at Pr. ≤0.05*

	w.P./w.Pt.	w.P./P-Pt.	w.Pt./w.B.	w.Pt./w.Ba.	P-Pt./l.C.	P-Pt./w.B.
Terrestrial vs. Aquatic	Pr. >0.05		Pr. >0.05	Pr. >0.05		
Terrestrial vs. Arboreal	Pr. >0.05	Pr. >0.05	Pr. >0.05		Pr. >0.05	Pr. >0.05
Terrestrial vs. Fossorial	Pr. >0.05		Pr. >0.05			
Aquatic vs. Arboreal	Pr. <0.01	Pr. <0.05	Pr. >0.05			
Aquatic vs. Fossorial	Pr. >0.05		Pr. >0.05			
Arboreal vs. Fossorial	Pr. <0.05	Pr. <0.05				

to follow, the variation between the groups will first be examined and then the variation within each group will be considered.

Character 1. w.P./w.Pt. The frequency distributions of the seventeen terrestrial and seventeen aquatic snakes in the sample were found to differ with respect to w.P./w.Pt. The H-test failed to reveal a significant difference in the central tendencies of these groups. An inspection of the frequency diagram for this character suggests that the difference disclosed by the Kolmogorov-Smirnoff test arises from a tendency of the aquatics to be skewed toward greater values for this character and the terrestrials skewed toward the lesser. The tendency is for the aquatics to possess prezygapophyses which extend farther beyond the postzygapophyses than this structure does in the terrestrial snakes.

There is evidence that the divergence disclosed here is the consequence of a biased sample. No significant difference between the aquatic colubrids and the terrestrial colubrids was found with respect to this character (table 5). There are more aquatic than terrestrial colubrids (10:4) in the sample. There is no significant difference between the Crotalidae of different modes of life and between the Crotalidae and Viperidae (see analysis of families) with reference to w.P./w.Pt. There are more terrestrial crotalids and viperids than aquatic (7:1) in the sample. In the analysis of the family classifications it was found that the Crotalidae and Colubridae were significantly distinct. Therefore, it seems quite likely that no real difference exists between the aquatic and terrestrial snakes taken *in toto* but that the frequency distribution of this character is controlled chiefly by taxonomic membership.

While this character does not distinguish the aquatic and terrestrial groups taken in their entirety, the extreme members of both of the classes apparently do exhibit a common adaptive modification. The high values of this character are found in snakes whose prezygapophyses are prominently extended laterally. In the very aquatic Homalopsinae, the sea snakes and the swiftest terrestrials, the prezyga-

pophyses are disproportionately long when compared with the majority of snakes. The implication is that in snakes in which the undulatory motion is exaggerated, this structure is so modified. Several of the important muscles employed in flexing the column laterally, and in moving the ribs, take origin directly or indirectly upon the prezygapophyses (Mosauer, 1935). Presumably, the lateral extension of this structure beyond the postzygapophyses will increase the efficiency of several important lever systems.

The frequency distributions of the 17 aquatic and the 14 arboreal snakes in the sample were found to differ with respect to w.P./w.Pt. (fig. 4). The H-test revealed a significant difference in central tendencies. In aquatic snakes the width of the prezygapophyses was found to be greater than the width of the postzygapophyses when compared to the arboreals.

The fact that a significant difference in central tendency was detected between aquatic colubrids and arboreal colubrids, suggests further that the observed differences are quite real in terms of these mode-of-life categories. The difference can again be attributed to the emphasis on the width of the prezygapophyses in snakes where there is a premium on undulatory movement. The low value obtained in arboreals cannot, however, be interpreted solely as any "de-emphasis" on undulatory activity for at least one other functional factor must be considered in the expression of this character. The short prezygapophyses of arboreals are apparently associated wtih the narrowness of body in these snakes as discussed more fully in connection with the following character.

Character 3. w.P./P-Pt. The frequency distributions of the seventeen terrestrial and fourteen arboreal snakes in the sample were found to differ with respect to the character w.P./P-Pt. (fig. 4). The H-test revealed a significant difference in central tendency, the terrestrials showing the higher values.

An examination of the radiographs disclosed that high values for this character are the consequence of vertebrae that are in proportion quite broad but short, the prezygapophyses extending out at nearly right angles to the vertebral body. Low values indicate narrower and rather elongated vertebrae. In terrestrials such vertebrae exhibit long prezygapophyses directed forward.

It is necessary to note that most of the arboreal boids do not depart from the family distribution with respect to the character w.P./P-Pt. The arboreal Crotalidae and the single arboreal viper appear to be shifted only slightly in the direction of elongation from their familial form. The arboreal and terrestrial colubrids do not differ significantly in the sample, although the aquatic and arboreal, fossorial and arboreal do (table 5). Although an adaptive trend is evident, it would appear that the distribution of this character is partially reflecting taxonomic control.

The trend within the arboreals revealed by the analysis is towards a narrow, elongated vertebra with short zygapophyses. This vertebral form is most extremely developed in the vine snake arboreal type. In these snakes, the body is slender and quite long. In contrast, the other arboreal type is characterized by a short, heavy body and a prehensile tail. Within this latter group the vertebral form exemplified by the vine snakes is approached in many instances.

The elongation of vertebrae is apparently related to the length of the body as well as width for this tendency was found among the long, and not particularly narrow, terrestrials (for example, *Pseudechis*). Increase in body length within a phyletic series of snakes apparently is accomplished by a general increase in size and/or elongation of the vertebrae without necessarily involving an addition of body segments. The number of vertebrae as given by the ventral scutes, was plotted against known maximum length for seventy snakes. The resulting scatter diagram indicates that body length is not highly correlated with the number of ver-

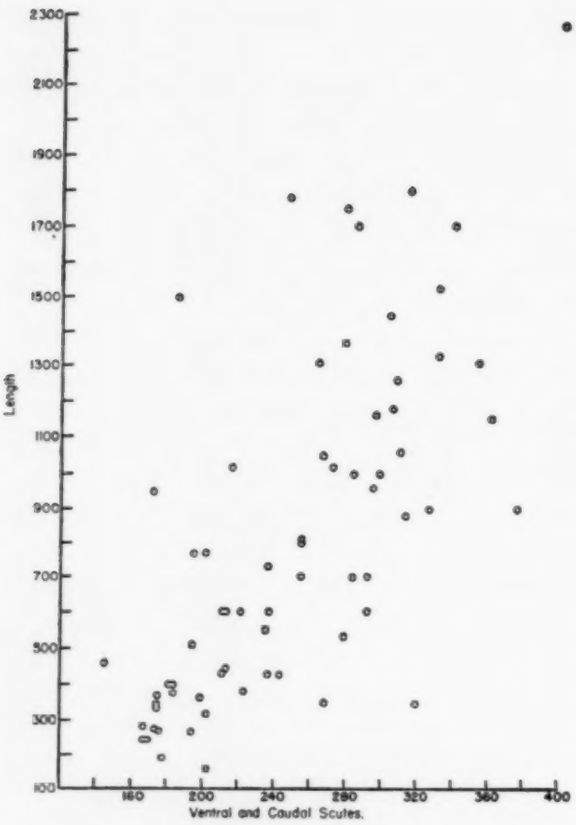

FIG. 5. Scatter diagram of length of snake against total number of ventral and caudal scutes. Total number of vertebrae equal to total number of scutes. (Data from Boulenger, 1893–96).

tebrae (fig. 5). This conclusion is dramatically emphasized by *Leptomicrurus* whose scant two feet includes as many vertebrae, over three hundred, as the giant pythons.

The body form of the vine snake type and the characteristic green or mottled coloring of these snakes, combine to render them almost invisible in their native haunts. Increase in length without a corresponding increase in body weight might be expected to be advantageous in bridging holds, but the narrow body appears to be more than the consequence of protective subterfuge and length without weight. The very arboreal boas, pythons and vipers are short and heavy bodied but laterally compressed.

In many of the heavy bodied forms the narrow body is not the result of short ribs and narrow vertebrae, but is accomplished in yet another manner. The ribs are directed downward rather than outward, producing a thin but very deep bodied animal (*Boa canina, Trimeresurus wagleri,* etc.). The boas in which the body is not normally compressed, distinctly narrow when extending the unsupported body towards a new hold. It would appear from these observations that the narrowness of body is associated with the ability of unsupported extension. Of interest in this connection is the fact that the terrestrial whip snakes are excellent climbers. An interpretation of the mechanical significance of this lateral compression must await a more thorough analysis of musculature and the functional requirements of the arboreal habitat.

In comparison with the arboreal vertebral form, the vertebrae of the majority of the terrestrials were found to be shorter, broader and bearing relatively longer prezygapophyses. The exceptions to such a configuration are *Thamnophis sauritus* and *Coluber*. These animals parallel the arboreals in the relative length of the vertebral body as expressed by the pre-postzygapophyseal distance. In contrast to the tree snakes, however, they possess prominent zygapophyses. The elongation of the vertebrae and the narrow vertebral body as seen in these snakes is again associated with a slender, whip-like body form. In his excellent analysis of the mechanism of locomotion in snakes, Gray points out that for very rapid progression, " . . . the curvature of the body should be such as to provide curves of long wave length and low amplitude" (Gray, 1946). Such curves are more readily thrown in a slender, flexible body than in the more stocky body form.

The frequency distributions of arboreal and fossorial snakes also were found to differ with respect to the character w.P./P-Pt. (fig. 4). The H-test revealed a significant difference in the central tendencies of these mode-of-life groups with the arboreals showing the lower values.

Since arboreal and fossorial colubrids were found to be significantly different, the distinction appears to be consistent

with the hypothesis of an adaptive difference (table 5). The fossorial snakes are quite similar to the aquatic and terrestrial snakes in this instance. Therefore, the divergence may again be ascribed to the specialization of the arboreal snakes as described above.

Character 4. w.Pt./w.B. The frequency distribution of the seventeen terrestrials and nine fossorial snakes in the sample were found to differ with respect to the characters w.Pt./w.B. (fig. 4). The H-test revealed that in the terrestrial snakes the relative width of the postzygapophyses to the width of the vertebral body was greater than in the fossorial sample.

No significant difference was found between fossorial and terrestrial colubrids (table 5). An examination of the frequency diagrams for the mode-of-life classes suggests that the tendency revealed here is not impressive (fig. 4). Since this character was later shown to be of taxonomic significance, the evidence for adaptive modification in this instance cannot be regarded as conclusive.

In spite of these objections, the difference in frequency distributions probably reflects a real, if slight, distinction between these groups. The postzygapophyses are more prominent in terrestrial snakes than in the burrowers. Since these structures are intimately involved in the important muscle linkages flexing the column laterally, the modification is probably associated with the requirements of rapid progression.

Character 5. w.Pt./w.Ba. The frequency distributions of aquatic and terrestrial snakes were found to differ with respect to the character w.Pt./w.Ba. (fig. 4). The H-test revealed a significant difference in their central tendencies. The difference disclosed by these tests arise from the tendency of the aquatic snakes to have a wider postzygapophyses relative to the width of the condyle than the terrestrial snakes.

Terrestrial and aquatic colubrids do not differ significantly with respect to this character (table 5). The aquatic and terrestrial sample suffer the same taxonomic bias found to affect the distribution of character 1, w.P./w.Pt. While the mode-of-life groups cannot be regarded in their totality as being distinguished by this character, the extreme members of both the classes apparently do exhibit a common adaptive modification.

The extremely high values were found in vertebrae in which the zygapophyses were relatively great and the condyle normal in width. In a few instances, however, the width of the zygapophyses was normal but the condyle was disproportionately small. Such vertebral forms were found among the very aquatic snakes and the swift terrestrials.

The association of prominent zygapophyses with undulatory activity was postulated earlier in the interpretation of several characters. Of special interest here, however, is the combination of these features with a relatively small condyle. As the size of the articulating ball is decreased, a reduction in resistance to turning in the socket might be expected. In the powerful terrestrial snakes the condyle is relatively enormous and must be a factor in increasing the strength of the column. Particularly this is true among the heavy bodied snakes where the column is subject to the great impact of the strike. In the water, there is less need for a shock resistant backbone and the condyle is partially freed to better accommodate the requirements of swimming. Whether or not such a mechanical advantage is realized, the association of the small condyle with undulatory facility is emphasized by the fact that the sidewinders, *Cerastes* and *Crotalus cerastes,* agree with the aquatic and swift runners in this detail. In doing so they are in sharp contrast to the rest of their kind.

From a survey of the frequency diagrams of all twelve characters it is clear that in the vast majority of snakes, vertebral form does not reflect gross mode-of-life. Even in the characters discussed above, the differences detected could often

be traced to the influence of a relatively small number of snakes showing extreme divergence. While the over-all picture emphasizes the stability of vertebral form in snakes, such a conclusion does not satisfy the objectives of this study. The rational application of these findings to fossil materials and the modern fauna requires an accurate assessment of adaptive significance. The details of whatever plasticity is evident, are in this context as important as the general inference of stability. Such detail may be lost as a consequence of an irrelevant classification of activity and habitat. A more accurate perspective might be achieved by examining the variation within each mode of life group.

Terrestrial snakes. The variation of the characters within the terrestrial sample is consistent with a subdivision of this grouping into three lesser categories: swift, general and heavy bodied terrestrials. In the main, the general terrestrial is indistinguishable from the majority of aquatic and fossorial snakes. The vertebral form of heavy bodied snakes is not sharply set off from the general terrestrials but shows certain consistent trends throughout the suite of characters. The swift terrestrials were found to be the most distinctive.

The swift snakes are characterized by narrow, elongated vertebrae with relatively long prezygapophyses directed forward. In addition to the instances earlier noted, these snakes could be distinguished from the other terrestrials by several of the other characters studied. Thus for example, a test of character 3, w.P./w.B., revealed a significant difference in central tendency between the swift and the remainder of terrestrial snakes. The constancy of agreement between these swift runners and the typical aquatic snakes throughout the series accentuates the essential similarity of their problems in locomotion.

Frequently the cobra *Walterinnesia* was found to be associated with the swift terrestrials. It is difficult to consider this snake as being functionally related to the group. The distribution of the Elapidae, however, indicates that the association is familial and so perhaps fortuitous. An alternative interpretation leaves us with the intriguing inference that the remnants of a primary elapid adaptation are disclosed in this affiliation of swift terrestrials, swimmers and the Elapidae. It is not inconceivable that the elapid whip snakes of Australia represent a primitive elapid body form.

In contrast to the swift snakes, the vertebrae of such forms as *Bitis* and the terrestrial crotalids tend to be stocky, with the zygapophyses nearly equal in length. The density of the bone as revealed by the x-rays, the robust appearance of the vertebrae and the relatively enormous condyles gives an impression of great columnar strength. In the relative overdevelopment of the articular structures, these snakes appear to be admirably equipped to deliver the strike. In general configuration the vertebrae of these powerful snakes resemble those of the Boidae.

Aquatic snakes. The variation within the aquatic sample appears to justify the recognition of the vertebral form of the best swimmers as an adaptive modification. The Hydrophidae and Homalopsinae were found to possess elongated vertebrae which bear prominent zygapophyses and relatively small condyles. The sea snakes, for the most part, could not be distinguished from the main body of the aquatics. A significant difference was obtained only with the character w.Pt./w.B. The genus *Natrix,* as represented by individuals of four species, was found to agree with the typical aquatic families. *Ancistrodon piscivorus, Chersydrus granulatus* and *Eunectes* seem to resemble their respective families rather than the other aquatics.

Arboreal snakes. The arboreal snakes emerged from the analysis as the most distinctive of the mode-of-life groups. In almost every instance the prezygapophyses were found to be relatively short, almost equal in length to the postzygapophyses,

and extending out at right angles to the vertebral body.

Two arboreal vertebral types, corresponding to the two arboreal body types, were found to be significantly different with respect to the character w.P./P-Pt. The vine snakes possess elongated vertebrae, narrow for the most part. The heavy bodied tree snakes tended to have relatively short and broad vertebrae agreeing more with their family distributions with respect to this character. The same difference was obtained in testing these body types with the character P-Pt./l.C., confirming the distinction in vertebral body length.

The most remarkable vertebral modification encountered in this study was found among the arboreal snakes with reference to the character w.Pt./w.B. Two types of arboreal snakes were evident in the distribution of this character. In group A the body of the vertebra is quite broad, while in group B it is normal, the post-zygapophyses being the same. These groups did not correspond with the arboreal body types.

Examination of the radiographs and cleaned skeletons revealed that the body width of group A snakes were broad as a consequence of a strong, laterally projecting shelf running between the zygapophyses. The taxonomic membership of this distinct group suggests this feature to be of adaptive significance:

Group A:
- All the arboreal Boidae (5)
- *Imantodes* (Colubridae)
- *Boiga* (Colubridae)
- *Trimeresurus wagleri* (Crotalidae)

Group B:
- *Dendrophis* (Colubridae)
- *Passerita* (Colubridae)
- *Oxybelis* (Colubridae)
- *Atheris* (Viperidae)
- *Trimeresurus schlegeli* (Crotalidae)
- *Dendraspis* (Elapidae)

The members of group B do not possess the very strong ridge, and agree with their family distributions in the main.

One possible interpretation of the shelving in group A suggests itself. In all snakes, the development of osteofascial tunnels through which the long muscles extend is another remarkable feature of this radiation. The most prominent of these tunnels hold the *M. semispinalis et spinalis* in place above the zygapophyseal ridge and against the vertebra medially (Mosauer, 1935). In this manner the muscle, which may extend over thirty vertebrae in its various linkages, is prevented from forming the chord of an arc in uni-lateral flexion. The lateral shelf, as developed in the extreme by *Imantodes* and *Chondropython*, provides an extensive bony floor to this tunnel. The habit of extending the unsupported body considerable distances in climbing from hold to hold requires that the otherwise highly flexible column be locked into a rigid beam. The simultaneous flexion of the long epaxial musculature would accomplish the desired rigidity, but any tendency for these muscles to be displaced downward would flex the column dorsally into a convex arc. The reverse arc is observed in tree snakes greatly extended towards new holds. The bony reinforcement of the floor of the osteofascial tunnel would be expected to prevent the downward displacement of these muscles.

All the Boidae possess a strong zygapophyseal ridge, though not all as pronounced as in the arboreal boids. If the interpretation of this shelf as given above is correct, then this feature would appear to be the ultimate in arboreal refinement. The interesting implication is that the primary radiation of the Boidae was arboreal.

Fossorial snakes. The fossorial snakes appeared to be identical with the majority of aquatic and terrestrial snakes. Within this group no divergence from familial patterns could be detected. Thus, it is concluded that there is no adaptive modification apparent among the fossorial snakes with regard to the characters studied. In other aspects, the burrowers do appear to share some common features. The vertebrae of these snakes are relatively small

when compared to body size, and the neural spines and hypapophyses are characteristically weak. In general the column appears to be reduced and, correspondingly, the trunk musculature is diminished in bulk. The mechanics of burrowing have not been studied but the morphological evidence suggests that the demands of this habitus upon the vertebral column are less rigid than in terrestrial life. Curiously enough, this inference may also be drawn from the number of vertebrae in the column of fossorial snakes. Above ground there seems to be strong selection upon the number of vertebrae within a column. Inger reports greater variability in vertebral number among juveniles than in the adults of *Thamnophis radix* (Inger, 1943). A similar observation was made by Dunn (Dunn, 1942). Among the burrowers, however, the variability of vertebral number is surprisingly great within species and among closely related species (for example, in the genus *Calamaria*). The possibility that this variability reflects a release from selection is further implied by the apparent reduction of the column.

Summary of the analysis of mode of life. In summary, it has been seen that in the vast majority of snakes, last moment specialization does not reach the level of vertebral modification. The adaptive tendencies highlighted by this analysis are expressed among a relatively few animals whose general body form and detail betray extreme adaptation to rather specific modes of life. This generalization is completely consistent with the findings of Mosauer in his study of the musculature of the trunk in snakes (Mosauer, 1935).

The anatomical features found to be the most subject to adaptive modification were the relative lengths of the zygapophyses, the relationship of vertebral body width and length and the relative size of the condyle. It is these variables that determine the general appearance of a snake vertebra in its dorsal aspect. In snakes in which a premium upon undulatory facility might be expected, the prezygapophyses were found to be prominently developed and the condyle reduced. The whip-like body form is reflected in elongation of the vertebrae and a narrow vertebral body. Heavy bodied snakes possess rather stocky vertebrae which bear relatively large condyles and zygapophyses of nearly equal length.

None of the mode-of-life groups were found to show complete internal consistency with respect to any of the characters studied. The arboreal snakes appear to be the most modified in vertebral form. Of particular interest in this group is the development of a bony shelf running between the zygapophyses. The burrowing snakes do not exhibit modification beyond familial boundaries. With respect to vertebral requirements, it would seem that this mode of life is the least demanding. This observation is fortified by the frequent development of burrowing forms throughout the major groups. The confirmed aquatics and swift terrestrials show analogous modifications presumably related to a similarity in locomotion. The marine and aquatic snakes were found to essentially agree in vertebral shape. In the sea snakes the adaptive modifications cited may also be regarded as of phylogenetic significance.

Now possessed with some insight into the gross adaptive significance of vertebral form, it is possible to approach the problem of phylogenetic significance. These two aspects of morphology are, in the last analysis, not mutually exclusive. To consider the phylogenetic significance of structure is to approach those subtle adaptations that in time long passed gave rise to the unique features of the taxon. In a real sense what is being compared here is the old and the new adaptive significance of vertebral form.

Analysis of Families

The Kolmogorov-Smirnoff test was performed for each character between each family for which a suitable number of observations was available. The relative

TABLE 6. *Characters involved in significant differences among the families*

	F_8	F_9	F_{10}	F_{11}	F_{12}
Boidae (F_4)	w.P./w.Pt. w.P./w.B. w.Pt./w.B. w.Pt./w.Ba. w.Pt./P-Pt. P-Pt./l.C. l.C./w.B. w.B./w.NC. w.Pt./l.C. P-Pt./w.B.	w.P./w.Pt. w.P./w.B. w.Pt./P-Pt.	w.P./w.Pt. w.P./w.B. w.Pt./w.B. l.C./w.B. w.Pt./l.C. P-Pt./w.B.	w.P./w.B. w.Pt./w.B.	w.P./w.B. w.Pt./w.B. l.C./w.B. w.P./l.C. P-Pt./w.B.
Colubridae (F_8)		w.P./w.B. w.Pt./w.B. w.B./w.NC.		w.B./w.NC.	w.P./w.Pt. w.P./w.B. w.Pt./P-Pt. w.Pt./l.C.
Hydrophidae (F_9)			w.P./w.B. w.Pt./w.B.	w.Pt./w.B. w.B./w.NC.	w.P./w.Pt. w.Pt./P-Pt.
Elapidae (F_{10})					w.P./w.Pt.
Viperidae (F_{11})					
Crotalidae (F_{12})					

integrity of the families so revealed is consistent with the thesis that vertebral form in snakes is of primary phylogenetic significance. Six families were analyzed: Boidae, Colubridae, Hydrophidae, Elapidae, Viperidae and Crotalidae.

Of the 180 comparisons of frequency diagrams made, forty instances were found in which there were significant differences between family pairs. Only the character w.P./P-Pt. failed to demonstrate a single difference. These results are in sharp contrast to the distributions of these characters as grouped by mode-of-life. The characters involved in these cases are shown in table 6.

The primary purpose of this study has not been to develop keys for the identification of isolated vertebrae, but to explore the meaning of vertebral form and in part to establish principles for further systematic treatment. Therefore, no attempt will be made here to define each family in terms of vertebral characteristics. Before such definition can be of real use, it is necessary to transfer the conclusions of the radiographic studies to the actual vertebrae and to utilize the additional features available from such material. General, but not diagnostic, descriptions of the vertebrae of many families are available elsewhere (de Rochebrune, 1880; Gilmore, 1938).

Inasmuch as the characters studied represent vertebral form, the number of differences found between families will be a measure of their similarity. Such a measure must be interpreted with extreme caution with regard to the apparent similarities involving the families represented in the sample by a few observations. Thus, the apparent resemblance between the Boidae and Hydrophidae is the implication of negative evidence and is certainly the consequence of the small (4) number of observations in the hydrophid sample. While this restriction severely limits the utility of the present sample,

certain of the results appear to be meaningful.

The Boidae emerged from the analysis as the most distinct of the families. The group was involved in twenty-six of the forty instances of difference encountered. No less than ten differences were found between this family and the Colubridae. Of special interest was the implication of the results that the Boidae resemble the solenoglyphs and proteroglyphs more closely than the Colubridae. The distinctiveness of this family is consistent with all other sources of phylogenetic evidence.

The Colubridae were responsible for eighteen of the differences encountered. The family could not be distinguished from the Elapidae and Viperidae. In spite of the small number of hydrophids and crotalids in the sample, the Colubridae differed quite distinctly from these groups.

The Elapidae were found to differ in nine respects, and to be indistinguishable from the Colubridae and Viperidae. The differences found between this family and the closely related Hydrophidae were concerned with the relative width of the zygapophyses, features which were earlier shown to be associated with an adaptive tendency in the sea snakes.

The Hydrophidae differed from each of the other families in at least two characters. Thus, in spite of the small sample, the positive results of the analysis serve to set this family apart from all but the Elapidae. The vertebrae of the sea snakes show other distinctive features not revealed by the characters studied. The radiographs of the lateral aspects of their vertebrae revealed excessively long neural spines distinctly longer in the crest than at the base. Presumably this feature reflects the deepening of the body in these snakes in their development of the remarkable oar shape.

The Viperidae were involved in only five instances and were not distinguishable from the Elapidae and Crotalidae. This result is hardly conclusive, however, since only four vipers were present in the sample. The Crotalidae, in contrast, were sharply set off from the other families differing in twelve circumstances in all.

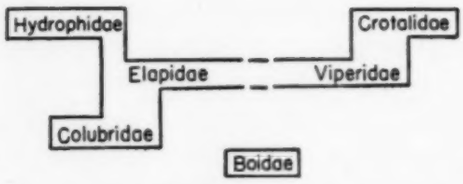

FIG. 6. Familial affinities as suggested by vertebral form.

These findings may be summarized as in Figure 6 if we assume each character to be as efficient in representing a distinct difference in vertebral form as any other.

In figure 6 adjacent families have been found to be indistinguishable in the analysis of the characters employed in this study.

The present sample size does not permit the acceptance of these results as conclusive data. Nevertheless one of the questions posed by this study requires that these findings be related to our present knowledge of the affinities of these families.

The present systematics at this taxonomic level are based chiefly upon evidence from the skull, teeth and scalation. This is unfortunate, since these anatomical features are especially subject to specialization. In recent years information bearing upon the phylogenetic problem has been gained from studies upon jaw musculature (Haas), trunk musculature (Mosauer), soft anatomy (Bellairs and Underwood etc.), hyobranchial apparatus (Smith and Warner), and pelvic relics. While the main features of the snake radiation have been recognized since Boulenger, the impact of these new sources of information has shaken the structure somewhat. The prevailing tendency has been to put off the inevitable synthesis and disregard the inconsistencies in the meanwhile. The nearest to such a synthesis has been provided by Schmidt in his summary, as a phylogenetic "family tree," of the families and sub-families (Schmidt, 1949).

The family Boidae is periodically split into two families, the boas and the pythons.

Morphologically such a split would appear to be unwarranted, although there is an argument on the adaptive side. The present study cannot contribute to this problem. Vertebral differences have not been found between the two pythons in the sample and the boas. Mosauer failed to find significant myological difference between the two groups (Mosauer, 1935). The distinctiveness of the Boidae in the vertebral analysis suggests that the group is quite old. While boids do not appear in the record until the Eocene, it seems likely that the family differentiated in the Cretaceous.

The critical region, from the standpoint of this study, involves the relationship between the families Colubridae, Elapidae and Viperidae. The consensus of the modern opinion seems to be that the Elapidae and Viperidae arose quite independently from a colubrid "pool." If the evidence from the vertebral analysis is taken at its face value, it implies that the Elapidae and the Viperidae are more closely related than the Viperidae and the Colubridae.

The several recent anatomical studies on these families have added critical data pertaining to their relationships. These studies have also revealed certain interesting inconsistencies. Thus, Mosauer reports that in back musculature, the Elapidae closely resemble the Colubridae but Acanthophis and the cobras show the viperid muscle arrangement. The other criteria formerly used to set off the elapine and viperine radiations are similarly compromised in many instances. These and other considerations prompted Bogert (1943) to postulate that the viperids were actually derived from a primitive elapid stock. Under the circumstances, the results of the vertebral analysis, as they pertain to these families, are not inconsistent with any conclusive evidence from other criteria.

There is no vertebral justification for the separation of the Viperidae and Crotalidae. Mosauer failed to reveal significant myological differences (Mosauer, 1935). The skull features and the occurrance of the pit in the Crotalidae do not appear to be associated with any distinct adaptive divergence. Wherever these groups meet the same environmental requirements, their reactions are almost identical as seen in the sidewinding and the aboreal adaptations. They are so similar in their mode of life as to not tolerate overlap. The recognition of these groups as distinct families on geographical distribution alone would tend to obscure the real phylogenetic picture. The geological record suggests that the Crotalidae were derived from the much older Viperidae, probably in the Pliocene (Gilmore, 1938).

It is concluded from the analysis of the family classifications that vertebral form in snakes, as expressed by the characters studied, is more closely associated with phylogenetic history than to adaptation to gross mode-of-life. The integrity of the genera *Crotalus, Natrix* and *Thamnophis* in the sample suggests further that vertebral configuration may be of use at even lower taxonomic levels than employed here.

The extensions of the conclusions and procedures of this study to the interpretation of the fossil record of the snakes would appear to be practical. Recognition of mode-of-life on the basis of fossil remains does not seem warranted in the main. In the very same stability that deters such an interpretation, however, rests the potential for the practical application of these findings to tracing of phyletic series.

The results of this study warrant the further exploitation of vertebral anatomy as a systematic tool. The great complex that is the Colubridae might be better understood through the application of evidence from this source. The statistical program and its logical framework could be adapted to a variety of other problems. Of particular interest is the possibility of utilizing these procedures in the study of the vertebrae of other animals in the geological record. The problems offered by such materials are not different from those

met here. Finally, the quantitative approach to the study of adaptation in morphology would appear to offer great promise.

Summary

The present study is a partial analysis of the variation in the form of the vertebrae of snakes and an attempt to associate particular patterns of variation with mode of life and phylogenetic lines. A sample of 57 snakes, representing 8 families and the classes terrestrial, aquatic, arboreal and fossorial, was employed in this study. Measures were obtained from radiographs and combined into ratios for the analysis. The characters studied were defined as the average ratios of pairs of measures from each of 10 vertebrae for every snake. The data were analyzed by the use of non-parametric statistical tests.

The results of the analysis of the mode-of-life categories indicated little association of the characters chosen for study and the four classes. The adaptive tendencies detected by the analysis were found to be expressed among a relatively few snakes, the vast majority remaining remarkably stable. The functional significance of the adaptive trends recognized was discussed.

The results of the analysis of the family classification revealed that vertebral form reflected taxonomic relationships to a greater degree than gross adaptation. Six families were analyzed and the results discussed with reference to phylogenetic significance. It was concluded that the extension of the procedures and findings of this study to the interpretation of the fossil record of snakes is practical.

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SELECTION IN *DROSOPHILA PSEUDOOBSCURA* IN RELATION TO CROWDING

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Introduction

When a population of *Drosophila pseudoobscura* containing less than 70 per cent of the Standard gene arrangement of the third chromosome and more than 30 per cent of the Chiricahua gene arrangement is introduced into a population cage at 25° C., the proportion of chromosomes with the Standard gene arrangement increases and the proportion with the Chiricahua gene arrangement decreases with successive generations (Dobzhansky, 1947). But chromosomes with the Chiricahua (CH) gene arrangement are not eliminated. Instead, the population reaches an equilibrium in which 70 per cent of the chromosomes are of the Standard (ST) gene arrangement and 30 per cent are of the CH type. This is due to selection which favors the heterozygote; the heterozygote is superior to both homozygotes and ST/ST is superior to CH/CH. These results were obtained with flies from the same Mendelian population at Piñon Flats, Mount San Jacinto, California. The increase in frequency of Standard chromosomes in the population-cage experiments simulates the increase in frequency of Standard at Piñon Flats in the summer. Standard is least common in June but its frequency increases during the summer and reaches a maximum in November (Wright and Dobzhansky, 1946).

The experiments described in this paper were designed to find out the stage or stages in the life cycle when selection occurred in laboratory populations at 25° C. and how selection was influenced by crowding. Crowding may be one of a number of components of selection and for any set of genotypes there is no *a priori* reason for supposing that it is always a necessary component. In view of the fact that most species are rare in relation to their necessary resources of space and food, most of the time, it would not be surprising to find that selective differences commonly exist between genotypes in quite uncrowded populations. (See Andrewartha and Birch, 1954.)

Three main series of experiments were done, each on a population having a low initial frequency of Standard (15 per cent to 30 per cent). The balance of the population consisted of flies with the Chiricahua gene arrangement. In one series of experiments adults and larvae were kept uncrowded from generation to generation. In another series, adults were crowded so that about 70 per cent died in the first two weeks of adult life, larvae being kept uncrowded. In the third series, larvae were crowded so that about 90 per cent of them died in the larval or pupal stage, the adults being kept uncrowded. With each successive generation the frequency of Standard and Chiricahua chromosomes was determined. Details of how these experiments were done are given in the section which follows.

Materials and Methods

Twelve strains of Chiricahua chromosomal types were mixed and the progeny of the mixture of flies were used for the initial populations of Chiricahua flies in

[1] The work reported in this article has been carried out during the tenure of a Fulbright Research Scholarship.

each experiment. The Standard flies used in the experiments were also derived from a mixture of 12 strains. Each strain was derived from a single female collected at Piñon Flats, Mount San Jacinto, California, in 1947 and since kept at 18° C. The Standard and Chiricahua flies were thus "genic heterozygotes." The food used was Spassky's medium with a suspension of Fleischman's yeast spread on the surface. Experiments were done in a constant temperature room kept at 25° C. Humidity in the room was not precisely controlled, but flies in all experiments were at a relative humidity close to saturation, since the bottles used contained a moist evaporating surface of filter paper. The frequency of chromosomes was estimated as follows. Eggs collected over a three-day period were placed in half pint milk bottles (40 eggs per bottle), with ample food. When the larvae were fully grown, their salivary glands were removed and stained with aceto-orcein. Each sample consisted of 150 salivary glands and consequently 300 chromosomes were examined.

A. Adults and larvae uncrowded

Four lots of 25 males and 25 virgin females up to 24 hours old were put each into a half pint milk bottle containing Spassky's medium to a depth of one inch and with yeast on the surface. This gave a ratio of 2.5 flies per square cm of food. Twenty per cent of the flies were homozygotes with the Standard gene arrangement, 80 per cent were homozygotes for the Chiricahua gene arrangement. The experiment was done in duplicate.

Twice a week for two weeks the flies were transferred to fresh bottles with fresh food. At the end of two weeks eggs were collected to start the next generation and for a chromosome sample. The procedure was as follows. Flies were transferred to bottles containing a small amount of food on paper spoons. Eggs laid on these spoons were collected at the end of 24 hours for each of three days. To start the next generation lots of 50 eggs were each put into a half pint milk bottle with food. Thus over the three-day period there were 12 bottles each with 50 eggs for each duplicate experiment. At this low density 97 per cent of the eggs gave rise to adults. When all adults had emerged, a random sample of 100 males and 100 females was taken from the 12 bottles and divided into four lots of 50 flies (half male and half female), each lot being put into a separate bottle. In this way both adults and larvae were kept at a constant low density of 50 flies per bottle. The procedure was repeated in successive generations, and the frequency of the three genotypes was determined in chromosome samples made in the third week of adult life of each generation.

B. Adults uncrowded, larvae crowded

Experiments were started as in A above with four bottles of 50 adult flies (20 per cent ST/ST and 80 per cent CH/CH) in each replicate. At the end of two weeks eggs were collected from these flies over a three-day period. These were used for starting the next generation and for chromosome analysis. Eggs were collected as follows. Flies were transferred to containers consisting of a glass cylinder (5½ cm internal diameter) closed at the base with the lid of a stender dish. The lid had an internal depression 4 cm in diameter into which had been poured Spassky's medium to a depth of 4 mm, and this was coated with yeast. The other end of the cylinder was stoppered with a cork. Within 24 hours the flies laid some hundreds of eggs in each container. The number of eggs was made up to or reduced to 400 in each container. Within three days it was usually possible to obtain 10 such containers each with 400 eggs and at the same time a sufficiency of eggs for analysis of chromosomes. After the eggs had hatched, strips of folded filter paper were pressed into the surface of the food; the paper was kept moist by the addition of a few drops of water each alternate day. It served to keep the chamber moist and also served as a site

for pupation. At this high density of flies, only about 10 per cent survived to become adults; the rest died from being overcrowded. As the survivors emerged, they were transferred to bottles with food. Four lots of 50 flies (half male and half female) were put into each of four bottles with food as described before. The procedure was repeated each generation. Not only did most of the flies die as larvae but those which survived took about 15 days to become adults as compared with 10 days in the case of flies which were uncrowded as larvae (experiment A). Chromosome samples were thus made every 5 weeks.

C. Adults crowded, larvae uncrowded

Adults were crowded to simulate the intensity of crowding in a population-cage. When numbers are high, about 4000 adults may be present in a population cage. Three hundred flies (half male and half female) up to 24 hours old, consisting of 20 per cent ST/ST and 80 per cent CH/CH homozygotes, were put into milk bottles with a small amount of food. The food had been poured, while liquid, into short lengths of glass tubing 1 cm deep with an internal diameter of 1.25 cm. This was held on a spoon and was replaced every alternate day. There was a ratio of 150 flies per square cm of fresh food which is about equivalent to the ratio in a population cage with 4000 flies. In terms of the number of flies per unit volume, the flies in this experiment were four times more crowded than flies in a population-cage. The crowding in these experiments was thus at least equivalent to that in a population-cage. The bottles also contained a pad of moist cotton which kept the air close to saturation. At the end of the 14th day an average of 72 per cent of flies had died.

On the fifteenth day eggs were collected from the survivors which had been transferred to fresh bottles, each supplied with a spoonful of food. It was necessary to use spoons for this purpose, as not enough eggs could be obtained from the small discs of food. Eggs collected were used for chromosome analysis and for starting the next generation. For this latter purpose 50 eggs were put into each of four bottles so that larvae were uncrowded. The experiment was duplicated. Since eggs were collected after about 70 per cent of the flies had died, they were laid when the flies were relatively uncrowded. Any differences between genotypes among eggs laid may reflect differences in adult death rate rather than differences in fecundity in crowded populations.

Results of Main Experiments

Results are summarized in table 1 and figures 1, 2, and 3. Although each experiment was started with a population of adult flies 20 per cent of which were homozygous Standard and 80 per cent of which were homozygous Chiricahua, the

TABLE 1. *The frequency (per cent) of Standard chromosomes in samples of 300 chromosomes examined in successive generations; (a and b are duplicate experiments). Figures in parentheses are mortality of adults per cent*

Generation	Adults uncrowded, larvae uncrowded		Adults uncrowded, larvae crowded		Adults crowded, larvae uncrowded	
	a	b	a	b	a	b
1	12.1	16.8	31.7	28.7	30.7 (65)	18.0 (70)
2	21.0	27.4	44.0	37.4	28.3 (67)	19.0 (70)
3	25.0	31.7	43.0	51.4	29.6 (84)	27.0 (75)
4	27.0	29.0	51.7	54.4	30.0 (72)	30.6 (69)
5	40.3	34.4	53.7	59.4	32.0 (73)	39.8 (72)
6	39.6	30.4	70.0	69.3	— —	— —
7	29.3	30.2	60.4	63.7	— —	— —
8	—	—	63.4	67.4	— —	— —
9	—	—	72.6	66.0	— —	— —

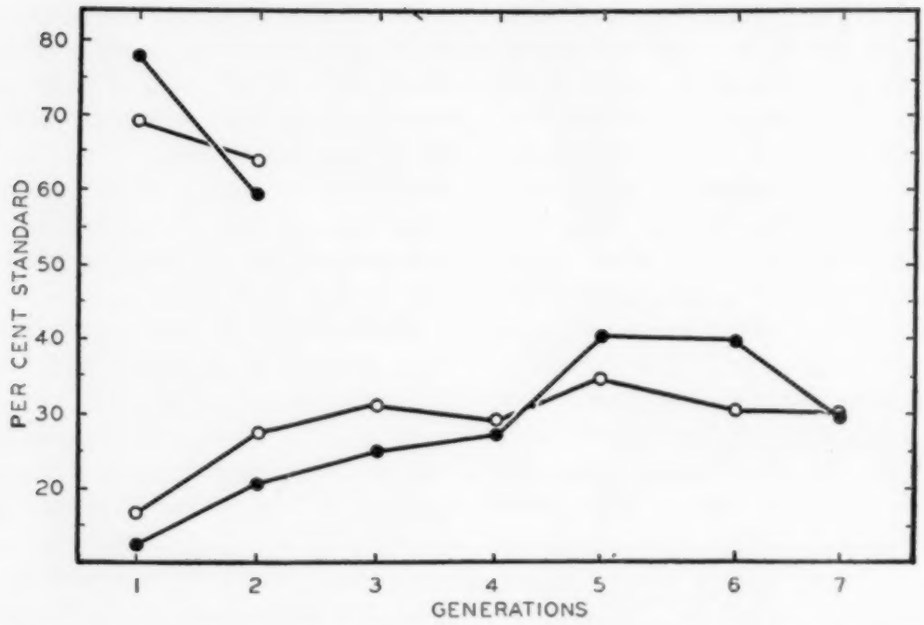

FIG. 1. The change in frequency of Standard chromosomes when adults and larvae were uncrowded. Open and closed circles refer to two replicates.

frequency of Standard in the first chromosomal count (from eggs laid in the third week of adult life) varied from 12 per cent to 30 per cent. This is probably due to differences between adults arising from differences in crowding of larvae from the initial cross of 12 strains. These differences do not, however, interfere with the general objectives of the experiments.

A. Adults and larvae uncrowded (fig. 1)

The frequency of Standard increased in both replicates to what appears to be an equilibrium at about 30 per cent Standard. Selection occurred at this low density of flies and was in favor of the chiricahua gene arrangement. It was not due to differential mortality of the flies since 97 per cent of eggs survived to become adults and practically none of the flies died in the first two weeks of adult life before eggs were collected. Selective differences evidently exist between adults in connection with mating and/or fecundity.

B. Adults uncrowded, larvae crowded (fig. 2)

The frequency of Standard rose steadily to the 6th generation (30 weeks). After this the frequency of Standard fluctuated about an equilibrium between 60 per cent and 70 per cent. Selection was in favor of the standard gene arrangement, and was clearly a result of crowding of larvae and probably a reflection of differential larval mortality of genotypes. The curve is remarkably similar to that obtained from population cages kept at the same temperature and in which Standard rose to an equilibrium of 70 per cent (Dobzhansky, 1947).

C. Adults crowded and larvae uncrowded (fig. 3)

The replicate which started at a frequency of 30 per cent Standard showed no change in the five generations of the experiment. But in the replicate which started with 18 per cent Standard, the frequency of Standard increased to 39 per cent in the 5th generation when the experiment had to be terminated. The trend of the latter curve is similar to that in experiments in which adults and larvae were both uncrowded. On the basis of this comparison and the existence of an equilibrium around 30 per cent Standard, it is reasonable to suppose that crowding of adults did not affect selection in these experiments.

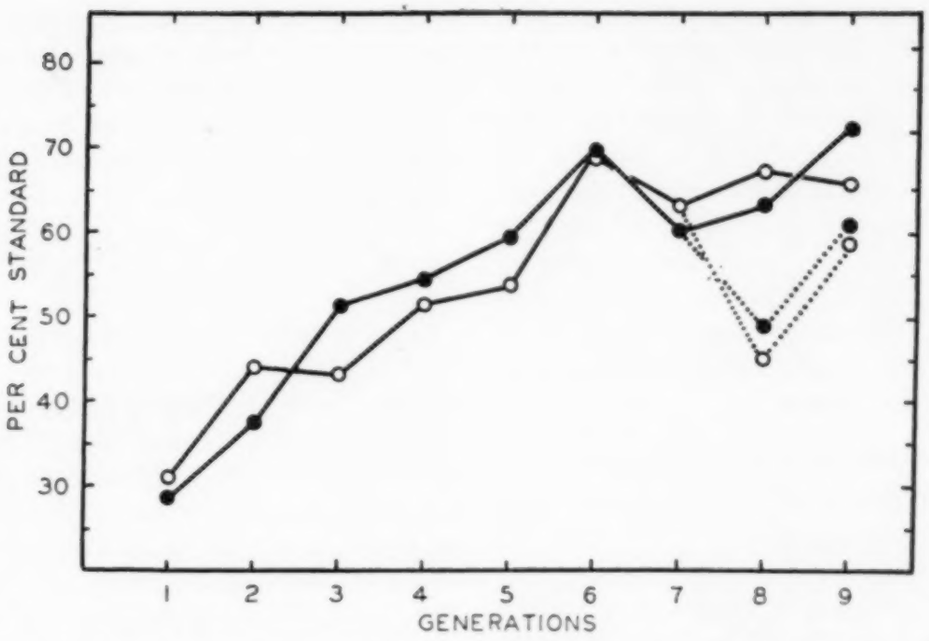

FIG. 2. The change in frequency of Standard chromosomes when larvae were crowded but adults were uncrowded (complete lines). Open and closed circles refer to two replicates. Dotted lines show change in frequency of Standard chromosomes when larvae and adults were both uncrowded from the 7th generation onwards.

The results of the three experiments may be interpreted as follows. When larvae were crowded so that 90 per cent died without becoming adults, there was rapid selection in favor of the Standard gene arrangement. Since an equilibrium was reached between 60 per cent and 70 per cent in the 6th generation, we may interpret these results in the same way in which Wright and Dobzhansky (1946) interpreted their findings in population-cages. The heterozygotes are superior to both homozygotes, and of the homozygotes ST/ST is superior to CH/CH. In other words, the selective coefficient of CH/CH is greater than that of ST/ST and the selective coefficient of ST/ST is greater than that of ST/CH. The selective coefficients were different when flies were less crowded, for when there were only 50 flies in a bottle, the frequency of Standard increased but only to an equilibrium of 30 per cent Standard. This shows that the heterozygote was again superior, but instead of ST/ST being superior to CH/CH, it is inferior. The selective coefficients will be in the order: ST/ST greater than CH/CH greater than ST/CH.

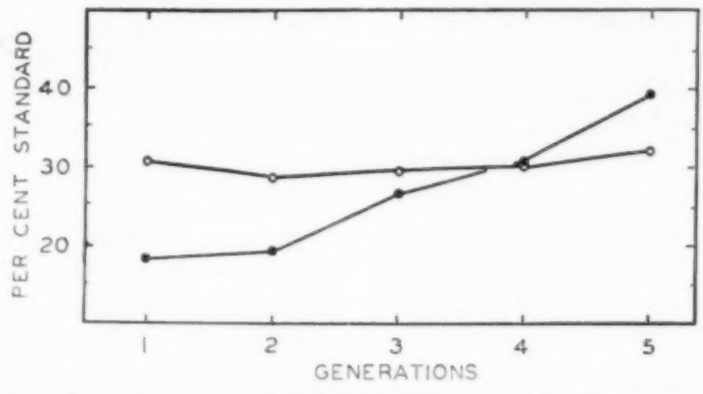

FIG. 3. The change in frequency of Standard chromosomes when larvae were uncrowded but adults were crowded. Open and closed circles refer to two replicates.

When larvae were crowded, selection was evidently due to differential mortality in the immature stages. This is not the case in the uncrowded populations since only 3 per cent of the flies died in the immature stages. There remain the possibilities that the genotypes differed in their sexual activity or in the number of eggs they laid. Differences in either of these characteristics could result in a change in frequency of chromosomes from generation to generation.

Confirmatory Experiments

Two additional experiments were done to provide confirmatory evidence that the adaptive values depended on density and that at a low level of crowding CH/CH was superior to ST/ST. After flies had been reared as crowded larvae for seven generations (fig. 2) and the frequency of Standard was 60 per cent in one replicate and 64 per cent in the other, eggs were collected and the larvae were reared uncrowded (50 per bottle). The experiment was then continued as in the original experiment with uncrowded larvae and uncrowded adults. There was a large increase in frequency of Chiricahua in the first generation (dotted lines, fig. 2) as compared with the controls (complete lines, fig. 2). In the second generation Standard increased in frequency but not to the level of the controls. Unfortunately the experiment had to be stopped at this stage for lack of time, so the results are no more than suggestions.

In a second confirmatory experiment, the procedure was identical with that in the experiment in which larvae and adults were kept uncrowded (experiment A) except that the initial ratio of Standard and Chiricahua was reversed. The initial population contained 80 per cent Standard and 20 per cent Chiricahua. The chromosomal frequency among eggs laid by these adults is shown in the top left corner of figure 1. In the first generation, the frequency of Chiricahua increased. This is shown by the sloping lines in the left top corner of figure 1. The experiment could not be continued beyond the one generation for lack of time and it should be repeated for a number of generations. Nevertheless the results suggest again the superiority of CH/CH to ST/ST in uncrowded populations.

Departure from the Hardy-Weinberg Ratios

The proportions of different genotypes in a Mendelian population will conform to the Hardy-Weinberg ratios when certain conditions are fulfilled. The conditions are that the different genotypes do not differ in sexual activity, that they mate at random, lay the same number of eggs, and provided the longevity of adults and the proportion of genotypes which die in the immature stages are the same for all genotypes. If the frequency of gametes carrying ST chromosomes is q and that of gametes with CH chromsomes is $(1 - q)$, the frequency of genotypes will be given by the expansion $(q + (1 - q))^2$ which is:

$$q^2 \text{ ST/ST} : 2q\ (1 - q) \text{ ST/CH} : (1 - q)^2 \text{ CH/CH}$$

Now, if the conditions stated above are not fulfilled, then the proportions of genotypes will depart from those predicted by the above expression. This statement is not absolutely general; for example, Hayman and Mather (1953) have shown that the Hardy-Weinberg proportions may be maintained, even under heavy selection, provided there are certain selective differences between genotypes. These particular cases must then be made provisos to the general rule.

Any of the differences between genotypes which cause departure from the Hardy-Weinberg ratio (other than non-random mating) will also cause a change in gene-frequency.

In the experiments described in this paper, we measured the frequency of genotypes among eggs laid, and from this estimated change in frequency from generation to generation. Consider the change in chromosome frequency first (figs. 1 and 2). In figure 2 this may be due to differential

mortality and/or the effect of intense crowding on sexual activity of adults or on the number of eggs laid by the different genotypes. The selection illustrated in figure 1 (uncrowded larvae, uncrowded adults) cannot be due to differential mortality as only 3 per cent of eggs fail to give rise to adults. It must be due to differences in the number of eggs laid by different genotypes or to differences in sexual activity or other similar cause operating in the adult, such as would cause a departure from the Hardy-Weinberg ratio among the eggs. In addition non-random mating would cause a departure from the Hardy-Weinberg ratio among genotypes (though this alone does not influence chromosome frequency). From the above argument we would expect to find departures from the Hardy-Weinberg ratio among genotypes of eggs laid in those experiments where larvae and adults were uncrowded. However, quite large differences between genotypes in any of these functions of mating and egg laying may cause only small departures from the Hardy-Weinberg ratio among genotypes, even though they may cause a large change in gene frequency. For example, consider a hypothetical population in which the frequency of Standard chromosomes is .50 and the frequency of Chiricahua is also .50. If flies mate at random and different genotypes lay the same number of eggs, the ratio of genotypes will be 1 ST/ST : 2 ST/CH : 1 CH/CH. Now let us suppose that Standard homozygotes lay twice as many eggs as either the heterozygote or Chiricahua homozygote. The ratio of genotypes will then become 3 ST/ST : 5 ST/CH : 2 CH/CH. The chromosome frequency of Standard is now .55 and that of Chiricahua .45. The ratio of genotypes expected on the basis of the Hardy-Weinberg rule is as follows:

3.025 ST/ST : 4.950 ST/CH : 2.025 CH/CH

Thus a doubling of the number of eggs laid by ST/ST has little effect in upsetting the Hardy-Weinberg ratio in the sub-

TABLE 2. *Frequencies of genotypes and of chromosomes with Standard (ST) and Chiricahua (CH) gene arrangements amongst eggs laid in experiments in which larvae and adults were uncrowded*

Chromosome frequencies, per cent			Genotype frequencies			
ST	CH		ST/ST	ST/CH	CH/CH	χ^2
12	88	Obs	4	28	118	
		Exp	2.2	31.7	116.1	
		Diff	+1.8	−3.7	+1.9	1.936
17	83	Obs	4	42	104	
		Exp	4.4	42.4	103.2	
		Diff	−0.4	−0.4	+0.8	0.046
21	79	Obs	6	51	93	
		Exp	6	49.5	93.5	
		Diff	0	+0.5	−0.5	0.008
27	73	Obs	12	58	80	
		Exp	10.8	59.2	80	
		Diff	+1.2	−1.2	0	0.157
25	75	Obs	7	61	82	
		Exp	9.3	56.2	84.5	
		Diff	−2.3	+4.8	−2.5	1.053
31.7	68.3	Obs	18	59	73	
		Exp	15.6	64.6	69.8	
		Diff	−2.4	−5.6	+3.2	1.241
27	73	Obs	11	59	80	
		Exp	10.8	59.2	80	
		Diff	+0.2	−0.2	0	0.002
29	71	Obs	10	67	73	
		Exp	12.6	61.8	75.6	
		Diff	−2.6	+5.2	−2.6	1.064
40	60	Obs	22	78	50	
		Exp	24	72	54	
		Diff	−2	+6	−4	0.963
34.4	65.6	Obs	18	67	65	
		Exp	17.7	67.6	64.7	
		Diff	+0.3	−0.6	+0.3	0.011
39.6	60.4	Obs	24	71	55	
		Exp	23.5	71.7	54.8	
		Diff	+0.5	−0.7	+0.2	0.019
30	70	Obs	18	54	78	
		Exp	13.5	63	73.5	
		Diff	+4.5	−9	+4.5	3.061
29.3	70.7	Obs	19	50	81	
		Exp	12.9	62.1	75	
		Diff	+6.1	−12.1	+6	5.722
30.2	69.8	Obs	10	71	69	
		Exp	13.6	63.2	73.2	
		Diff	−3.6	+7.8	−4.2	2.137

TABLE 3. *Frequencies of genotypes and of chromosomes with Standard (ST) and Chiricahua (CH) gene arrangements amongst eggs laid in experiments in which larvae and adults were uncrowded and when the change in chromosome frequency from the preceding generation was large (dotted lines fig. 2)*

Chromosome frequencies, per cent, of previous generation		Chromosome frequencies, per cent		Genotype frequencies				
ST	CH	ST	CH		ST/ST	ST/CH	CH/CH	χ^2
60.4	39.6	49.4	50.6	Obs	44	60	46	
(fig. 2)				Exp	38.6	76	35.4	
				Diff	+5.4	−16	+10.6	7.297
63.7	36.3	45.4	54.6	Obs	47	42	61	
(fig. 2)				Exp	45	74.4	30.6	
				Diff	+2	−32.4	+30.4	45.211
78.0	22.0	59.3	40.7	Obs	58	60	32	
(fig. 1)				Exp	53	72.5	24.5	
				Diff	+3	−12.5	+8.5	5.274
69.0	31.0	64.0	36.0	Obs	23	62	65	
(fig. 1)				Exp	19.5	61.5	69	
				Diff	+3.5	+0.5	−4	0.863

sequent generation, though the frequency of Standard chromosomes is increased by 5 per cent. Even if differences in egg laying exist, we may not be able to pick these up by examining genotype frequencies and calculating Hardy-Weinberg ratios. The same argument will, of course, apply to differences in sexual activity. Table 2 provides us with a concrete illustration. The increase in frequency of Standard chromosomes which is shown in this table is not, as already explained, due to differential mortality. The change in frequency from generation to generation is not associated with departure of genotype frequency from the Hardy-Weinberg ratio. Without exception the observed and expected frequencies are not significantly different.

These data show that selective differences among adults in sexual activities or in egg-laying, of the order found in these experiments, may cause little or no disturbance to the Hardy-Weinberg ratios, though influencing considerably chromosomal frequency. Consider next, however, the frequency of genotypes when the change in frequency of chromosomes with successive generations is much greater than in these experiments. The data associated with the fall in frequency of Standard in the first generation of the two confirmatory experiments provide examples of this. In three of the four replicates the frequency of Standard fell by 20 per cent in one generation. In each of these experiments, departure of the frequencies from the Hardy-Weinberg ratios was large and significant (table 3). But in the fourth replicate (lower line, table 3) the fall in frequency of Standard was only 5 per cent and in this experiment the observed proportions agree with those expected from the Hardy-Weinberg ratio. Evidently selective differences in sexual activity or fecundity were great enough in three of the four cases to cause a drastic disturbance to the Hardy-Weinberg ratios.

Selection which results in an equilibrium frequency of Standard in these and in other experiments is evidence of heterosis or superiority of the heterozygote. In addition to such evidence from experimental populations, there is direct evidence from the study of Hardy-Weinberg ratios in natural populations. Dobzhansky

and Levene (1948) showed that among adult males found in natural populations of *D. pseudoobscura,* the heterozygotes ST/CH were more and the homozygotes ST/ST and CH/CH were less frequent than required by the Hardy-Weinberg rule. Since, in these same populations, eggs were laid in proportions which conformed to Hardy-Weinberg ratios, it follows that differential mortality occurred sometime between the egg and adult stages. Epling, Mitchell and Mattoni (1954) showed that the relative frequencies of homozygotes and heterozygotes among adults of *D. pseudoobscura* collected at Piñon Flats conformed to Hardy-Weinberg ratios at some times of the year. But this does not prove there were no selective differences between the genotypes at such times. As already pointed out, quite large selective differences between adults in sexual activity and fecundity may cause only slight disturbance to the Hardy-Weinberg ratio. In addition to this, there are certain circumstances in which selective differences in mortality rates are not reflected in disturbance to the Hardy-Weinberg ratio. Departure of adult ratios from the Hardy-Weinberg ratios does, of course, demonstrate that selection has occurred. This has been shown for other species besides *D. pseudoobscura.* In natural populations of *D. paulistorum* and *D. willistoni* in Brazil, heterozygotes for two X-chromosome inversions were found to be in excess of proportions predicted from the Hardy-Weinberg ratio (Da Cunha 1953).

Implications for Natural Populations

Dobzhansky and Wright (1946) pointed out that the increase in frequency of Standard chromosomes in population cages at 25° C. was analogous to the rise in frequency of Standard and the decrease of Chiricahua in summer in California. The increase in Standard in experiments with crowded larvae described in this paper is likewise analogous to the changes which occur in the natural population in summer. During spring, however, the frequency of Standard falls and that of Chiricahua rises. This order has not been reproduced in experiments with population-cages, but this is just what happens in experiments in which larvae and adults are kept uncrowded. It is a reasonable hypothesis to suppose that this experiment reproduces the type of selection which occurs in spring in favor of Chiricahua. At this time of the year, food may be more abundant than in summer, populations may be sparse after the winter, so that larvae may well be uncrowded in relation to resources. In any case we have in the experiment with uncrowded flies an example of a population in which the change in frequency of Chiricahua is in the same direction as that in the field in spring. It may thus provide the necessary additional clue to interpreting the annual sequence in frequency of Standard and Chiricahua in the field.

Summary

1. Experiments were done with *Drosophila pseudoobscura* at 25° C. to find out (a) the stage or stages in the life-cycle when selection occurred in experimental populations and (b) the extent to which selection was influenced by crowding. Initial populations contained flies of which 20 per cent were homozygous for the Standard gene arrangement of the third chromosome and 80 per cent were homozygous for the Chiricahua gene arrangement of the third chromosome. In one series of experiments, adults and larvae were kept uncrowded (50 flies per half pint milk bottle) from generation to generation. In another series, adults were crowded so that about 70 per cent died in the first two weeks of adult life; the larvae were kept uncrowded. In a third series, larvae were crowded so that about 90 per cent of them died before the adult stage; the adults were kept uncrowded. Two subsidiary experiments were done with flies which were kept uncrowded, but in which the initial population contained a

high frequency of Standard chromosomes and a low frequency of Chiricahua.

2. When adults and larvae were kept uncrowded, the frequency of Standard chromosomes rose from about 15 per cent to an equilibrium of 30 per cent Standard. Selection occurred at this low density of flies, and the equilibrium value shows that CH/CH homozygotes were superior to ST/ST homozygotes. The existence of an equilibrium frequency demonstrates the superiority of the heterozygote to both homozygotes. Selection was not due to differential mortality among the genotypes, as only 3 per cent of them died in the immature stages and eggs were collected before any of the adults had died. The selective differences were evidently associated with mating and/or fecundity of adult flies.

3. When adults were uncrowded but larvae were crowded, the frequency of Standard rose steadily to the 6th generation. After this, it fluctuated about an equilibrium between 60 per cent and 70 per cent. Selection at the high density in these experiments was in favor of Standard. Again the heterozygote was superior to the homozygotes. The selective differences were probably due to differential mortality among the larvae.

4. When adults were crowded and larvae were uncrowded there was an increase in frequency of Standard to an equilibrium of about 30 per cent Standard. Crowding of adults evidently did not affect selection in these experiments even though 70 per cent of the adults died in the first two weeks of adult life.

5. The findings of the main experiments show that selection is a function of density, Chiricahua being favored by low density of larvae and Standard being favored by a high density. Further evidence for the superiority of Chiricahua at the low density was the increase in frequency of Chiricahua when flies which had been crowded as adults for seven generations were reared as uncrowded larvae. Likewise there was an increase in the frequency of Chiricahua when the initial population contained about 80 per cent Standard chromosomes and the larvae were reared uncrowded.

6. Selection (other than selective mortality) which was sufficient to cause an increase in frequency of Standard from about 15 per cent to 30 per cent in four generations, when larvae and adults were reared uncrowded, did not result in any significant disturbance to the Hardy-Weinberg ratios of genotypes among eggs laid. But when these selective differences were so great as to cause a change in frequency of Standard of from 15 to 25 per cent in one generation, there were highly significant disturbances to the Hardy-Weinberg ratios among genotypes of eggs laid.

7. Selection favoring Standard in experiments with crowded larvae may be analogous to the increase in frequency of Standard which is known to occur at Piñon Flats, California, in the summer. Selection favoring Chiricahua in experiments with uncrowded larvae may be analogous to selection in the spring at Piñon Flats when Chiricahua increases in frequency. The experiments with uncrowded larvae may thus provide an additional clue to interpreting the seasonal sequence in frequency of Standard and Chiricahua in the field.

Acknowledgments

It is a pleasure to acknowledge the unstinting help and advice given me by colleagues in the Zoology Department of Columbia University. The work was planned and executed with the continuous advice and encouragement of Professor Th. Dobzhansky who extended to me the generous hospitality of his laboratory. To him I am especially indebted. Dr. Richard Lewontin suggested some of the experimental methods which proved to be effective and I owe much to discussions with him. Dr. L. Levine taught me how to identify chromosomes and he generously checked many of my identifications. Both he and Dr. Betty Moore attended to cultures and experiments during

my absence from the laboratory on a number of occasions. Mrs. O. Pavlovsky taught me the art of making smears of salivary glands. Mr. Richard Tombaugh did the computations of chi-squares in Tables 2 and 3 and assisted with making the graphs. The manuscript was read and criticized by Professor Th. Dobzhansky, Professor J. A. Moore and Dr. Betty Moore of Columbia University and Dr. P. M. Sheppard of Oxford University. Without the assistance of all these workers, the work could not have been completed in the time available.

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A STUDY OF POPULATION GENETICS ON THE POLYMORPHIC LAND SNAIL *BRADYBAENA SIMILARIS* [1]

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INTRODUCTION

Land snails have been extensively used by previous authors as materials for the studies in population genetics and in the mechanism of evolution. This is mainly because many of them show high intraspecific variations in color and banding; moreover they have only a limited power of locomotion, and their lives much depend on environmental conditions, they are accordingly suited for the study on the effect of isolation on the differentiation of varieties and local races. The classical work by J. Gulick on Achatinellidae on Oahu, Hawaiian Ids. dates back to 1905. This family has since been studied by Pilsbry et al. (1912) and Welch (1938). Crampton investigated on another variable land snail *Partula* spp. inhabiting islands in the South Pacific (1916, 1925, 1932; also Lundman, 1947). In spite of the great labors taken for these studies, however, all these works on tropical land snails have now become out of date, primarily owing to the deficiency of the basic genetic data and inadequacy of the methods used for the analysis of the samples.

Of the land snails found in the temperate zones, the European wood and garden snails, *Cepaea nemoralis* and *C. hortensis* have been more frequently used as materials for genetic studies than any other species. Breeding experiments on these snails were performed first by Lang (1904, 1908) and subsequently by Stelfox (1918), Fisher and Diver (1934) and Lamotte (1951b). Natural populations of these snails were worked on by a number of investigators on zoogeography, evolutionary mechanism and population genetics. These works have been reviewed repeatedly, more recently by Lamotte (1951b), Sheppard (1953) and Cain and Sheppard (1954). Opinions are varied as to the cause of geographic and seasonal variations in the incidence of the genes governing the colors and bandings among different populations of these species: some authors (Diver, 1940; Lamotte, 1949, 1951 a, b, 1952) maintain the importance of the effect of genetic drift on these variations, while other authors (Boettger, 1931; Schnetter, 1950; Cain and Sheppard, 1950, 1952, 1954, and Sheppard, 1951, 1952, 1953) attribute them primarily to natural selection exerted by predatory animals. There is also much discussion with regard to the mechanism of maintenance of the polymorphism in these species: the British authors advocate Fisher-Ford's theory of balanced mechanism of selective agencies, while Lamotte attaches importance to the effect of recurrent mutations, as well as of migration. It seems rather strange that these authors using the same material should have arrived at conclusions so diverse from each other. This may be due, at least in part, to the difficulties inhalent in the material itself.

Bradybaena similaris apparently has some advantages to *Cepaea* as the material for this kind of study, in its smaller size and faster development. Furthermore, the polymorphism in this species is genetically simpler and more clear-cut than in *Cepaea*. The breeding experiments of this snail were carried out by Emura in 1931 through 1938. More recently, Komai took the material up for his studies on

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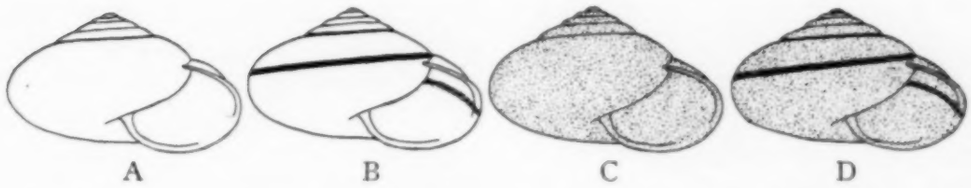

FIG. 1. *Bradybaena similaris.* Diagrammatic figures showing the polymorphism: A – YU, B – YS, C – BU, D – BS.

population genetics. The present paper deals with the conjoint results of our works.

THE MATERIAL

Bradybaena similaris Férussac is a land snail very small for a species belonging to the Family Helicidae, being 18 mm in diameter and 12 mm in height at most of its size. It has four distinct color and banding types, namely, 1. of yellowish color and without band (designated as YU), 2. of yellowish color and with a band of chestnut color (YS), 3. of brownish color and without band (BU) and 4. of brownish color and a band of chestnut color (BS) (fig. 1). As will be shown below, these four types are probably controlled by two sets of very closely linked genes which behave actually as triple-allelic genes. Of the various terminologies proposed for the genes in such relation—"semi-allelic" genes (Komai, 1950), "pseudo-allelic" genes (several authors) and "para-allelic" genes (Laughnan, 1952), the last one seems to be the best, and we shall use this terminology in subsequent descriptions.

This snail is widely distributed in the tropical and temperate coastal regions of Asia; it also occurs in some parts of Africa, Australia and Central and South America. It is considered to be indigenous in Southeastern Asia, and that unconscious human intervention was responsible for its present distribution. According to Pilsbry *et al.* (1912) and Gude (1914), the distribution has followed the cultivation of sugar cane.

In Japan this snail is found on the main islands excluding Hokkaido. Although its occurrence in the latter island has been reported (by von Möllendorff, 1885), this report needs confirmation. The snail also occurs on the smaller islands including Hatizyôzima and Titizima and Hahazima of the Bonin Group and on Okinawa. It has been collected at various localities in Formosa as well. The occurrence of the snail is rather fortuitous, and it is absent in many places where all environmental conditions appear favorable for its life. In some localities it is found only in small numbers. All these are apparently due to the fact that the snail was introduced into Japan relatively recently, and also that its range is restricted by the animal's limited power of locomotion and high susceptibility to environmental conditions. In fact, even a paved road a few meters wide, or a wall barely one meter high, seems to become a complete barrier to the range of this snail.

Among the taxonomists who have described the specimens of this snail from Japan, Arthur Adams (1868) collected specimens in 1861 at Osima in Wakayama Prefecture, as well as in Nagasaki and Simonoseki; von Martens (1877) reported it from Tokyo, Hakoné and Mt. Kano; Kobelt (1879) mentions that Rein collected this snail in abundance in Yokohama, and von Möllendorf (1885) records it from Poronai in Hokkaido. Among the old native naturalists, Hôbun Mizutani of Nagoya (1836) illustrates, in his catalogue of an exhibition of natural curiosities held in that city, a snail which is very likely this species, with a description, "whitish, with a pinkish band; found in Nagoya." This seems to be the oldest record of this snail occurring in Japan. There is no means of ascertaining exactly how long this species has been living in

Japan. If it was brought into this land with the sugar cane, as Pilsbry *et al.* suppose, it has been in Japan barely 200 years. Probably it was introduced several times, each time into a new place. It is possible there were many chances for this common and small snail to be introduced into a new habitat either by humans or by some other agency.

The snail is commonly found in vegetable gardens, or on sheltered grounds between houses covered with organic debris and it does some damage to vegetables with soft foliage like cabbage and sweet-potato plant. The active season extends from early spring to late autumn. Emura (1932) has described in detail the anatomy and ecology of this snail. The copulation takes place only between individuals carrying shells with reflected lip. It is reciprocal and differs in no way from the copulation in other allied land snails. It is repeated several times during the season. The frequency of oviposition and the number of eggs laid each time vary considerably. According to Emura's observation, the frequency of oviposition was eleven times on the average in one season, and from nine to twenty eggs were deposited each time. Some individuals kept in isolation were found to lay a few eggs apparently by self-fertilization, and some of these eggs developed to maturity.

The embryonic development in summer season takes from fifteen to twenty days. In cooler seasons the period extends to more than a month, or even as long as three months. The newly hatched snail carries a shell with 1.5 to 2 whorls and is 2.0 – 2.5 mm in diameter and 1.0 – 1.5 mm in height. The growth after emergence is rapid for a snail. In about a month the shell of the young snail, which is 2.5 – 3-whorled, has grown to about 4 mm in diameter, and bears the color and banding proper to each individual. In 70 days or so, the snail reaches the adult stage, which can be recognized by the reflected lip of the shell. Thus, it takes altogether 100 days or somewhat more from emergence to maturity.

According to our observations in the field and laboratory, this snail has many natural enemies, such as isopods *Armadillidium* and *Porcellio,* larvae of bettles, mites, nematodes, land planarians and infusorians. Of vertebrate enemies, our observations are rather deficient. Field mice and toads seem to do some damage to the snail population. The importance of birds as enemies of the snail is apparently relatively small. This view is supported by the kind communication of Dr. N. Kuroda, our expert in bird's ecology. He asserts that Japanese birds belonging to the genus *Turdus* do eat snails. These, however, form only a fraction of their food. It is doubtful if the visionary selection by these birds plays an important role in determining the relative incidence of the color types in this species, especially since the snails are usually concealed under foliage or some other objects. The mites, nematodes and infusorians are chief pests of young snails reared in the laboratory.

Breeding Experiments

The snail is easy to breed in laboratory. For individual cultures, glass cylinders 11 cm in height and 11 cm in diameter were used. For mass cultures, larger vessels, 24 cm × 12 cm were preferable. These cylinders had glass covers, and the bottoms were covered with sterilized sand kept in suitable moist condition to the depth of about 5 cm. Sliced cucumbers or the fruit of another cucurbit *Sechium* (= *Cicyos*) *edule* served as food. Grated carrots were used as substitute when the cucurbits were out of season. Powdered oyster shell provided the snails with their shell material. The snails to be used for breeding should be isolated before maturity. The eggs are laid in clusters in the deep layer of the sand. They are collected from the sand, and each cluster is put in a Petri dish with moist filter paper. The snails are raised in the dish until they are large enough to be raised on sandy substratum.

TABLE 1. *Results of breeding experiments*

Exp. no.	Type of mating	Number of mothers	F1				Total	x^2	DF	P
			YU	YS	BU	BS				
1	YU×YU	39	2103	0	0	0	2103	—	—	—
2a	YU×YS	18	*2*	1174	0	0	1176	—	—	—
b	YS×YU	19	0	1102	0	0	1102	—	—	—
3a	YU×YS	9	275	271	0	0	546	.029	1	.8 –.9
b	YS×YU	9	343	357	0	0	700	.028	1	.5 –.7
4a	YU×BU	13	*9*	0	773	0	782	—	—	—
b	BU×YU	12	0	0	901	0	901	—	—	—
5a	YU×BU	5	224	0	211	0	435	.388	1	.5 –.7
b	BU×YU	5	309	0	309	0	618	.000	1	1
6	YS×YS	14	0	1017	0	0	1017	—	—	—
7	YS×YS	32	642	2129	0	0	2771	4.957	1	.02–.05
8a	YU×BS	9	*12*	417	426	0	855	.096	1	.7 –.8
b	BS×YU	9	0	431	419	*1*	851	.169	1	.5 –.7
9a	YS×BU	3	0	0	0	271	271	—	—	—
b	BU×YS	3	0	0	0	383	383	—	—	—
10a	YS×BU	2	0	65	0	91	156	4.334	1	.02–.05
b	BU×YS	2	0	131	0	132	263	.004	1	.95–.98
11a	YS×BU	5	*1*	0	304	265	570	2.673	1	.1 –.2
b	BU×YS	5	0	0	224	243	467	.773	1	.3 –.5
12a	YS×BU	10	267	264	253	259	1043	.432	3	.9 –.95
b	BU×YS	10	251	251	261	281	1044	2.297	3	.5 –.7
13a	YS×BS	6	*1*	249	133	155	538	4.635	2	.05–.1
b	BS×YS	6	0	342	174	171	687	.039	2	.98–.99
14a	YS×BS	7	0	329	0	323	652	.055	1	.8 –.9
b	BS×YS	7	0	374	*1*	366	741	.086	1	.7 –.8
15	BU×BU	14	0	0	1282	0	1282	—	—	—
16	BU×BU	10	235	0	639	0	874	1.661	1	.1 –.2
17a	BU×BS	4	0	0	159	188	347	2.424	1	.1 –.2
b	BS×BU	4	0	0	160	178	338	.958	1	.3 –.5
18a	BU×BS	6	0	231	369	198	798	7.241	2	.02–.05
b	BS×BU	6	0	138	293	140	571	.408	2	.8 –.9
19	BS×BS	10	0	235	250	412	897	6.420	2	.02–.05
20	BS×BS	6	*1*	130	123	228	482	1.543	2	.3 –.5
21	BS×BS	14	*1*	248	238	465	952	.679	2	.7 –.8
22	BS×BS	10	0	171	167	337	675	.049	2	.95–.98
23	BS×BS	58	*1*	130	102	238	471	3.149	2	.2 –.3
24	BS×BS	76	0	234	257	522	1013	1.993	2	.3 –.5
25	BS×BS	60	0	152	177	342	671	2.115	2	.3 –.5
26	BS×BS	122	0	348	382	705	1435	2.047	2	.3 –.5
27	BS×BS	—	0	121	118	178	417	8.966	2	.01–.02

The experiments designated by the same number and a, b are those with the same parent pair. The italicized F1 progeny are unexpected types.

Emura began breeding the snail in 1931, and carried the stock through 1938. He published a part of the results, obtained by 1937, under the joint authorship with the late Dr. K. Ikeda (Ikeda and Emura, 1937). Later, in 1952, he published almost all of the data in concise form in the popular journal of genetics "Iden." More recently, we bred in our respective laboratories, the brown-banded types to get young specimens of known genotypes for the experiments reported in the present paper.

The breeding data are presented in table 1. These data apparently indicate that the four color and banding types in this snail are controlled by a set of triple-allelic genes. This interpretation was given first by Ikeda and Emura (1937). There are a few data that show that the

observed ratios diverge from the expected ratios slightly beyond the level of significance ($0.05 > P > 0.02$) (Nos. 7, 18a, 19). These divergences, however, are in various directions, and there is no uniformity among them. The culture no. 27 shows a greater lack of conformity to the expected ratio ($0.02 > P > 0.01$). This culture suffered from serious infections of mites, and should be considered abnormal.

Some cultures produced a few exceptional progeny, italicized in the table. Most of these exceptions seem to be the products of self-fertilization of the respective mothers. Only 3 exceptions, one in each of the cultures 21, 22 and 24, can hardly be attributed to this cause. Various explanations are possible for their origin, such as somatic mutation, incomplete penetrance of the dominant gene, or mere contamination.

In short, the breeding data as a whole conform very well to the interpretation given above. Question still remains as to whether these genes are allelic in the strict sense, or they are "para-allelic" sets. Although no conclusive evidence is available as yet, the latter view is more probable by reasons to be presented later.

Physiological Differences Among Genotypes

Growth rates and susceptibilities to low temperature were examined to determine whether or not there are any physiological differences among the different types. For simplification and ease in analyze, materials were chosen according to genotype instead of phenotype. Thus, only the progeny of the matings of brown-banded parents were reared. They should include brown-unbanded (BU), brown-banded (BS) and yellow-banded (YS) individuals in the ratio 1 : 2 : 1. Of these the BU and YS members are homozygotes. In gene symbols they may be designated as c^Bs/c^Bs and cs^+/cs^+ respectively, c standing for color which is yellow and s for banding or stripe; the brown-banded members are c^Bs/cs^+. The other genotypes, which are missing here, are cs/cs (double recessive yellow-unbanded YU), c^Bs/cs (heterozygous brown-unbanded) and cs^+/cs (heterozygous yellow-banded).

Table 2. *Comparison of sizes between the genotypes cs^+/cs^+, c^Bs/c^Bs and c^Bs/cs^+ at the stage with the shell diameter 3–6 mm. The size has been measured with an ocular micrometer of which one unit corresponds with .05 mm*

	Genotypes			
	cs^+/cs^+	c^Bs/c^Bs	c^Bs/cs^+	Total
Number of individuals	239	300	489	1028
Mean diameter	94.4204	95.7147	103.4356	
Source of variation	D.F.		S.S.	
Genotype	2		3,564.0389	
Replication	74		68,027.2105	
Error	148		37,689.5956	
Total	224		109,280.8450	
S.E. for mean	$\sqrt{\frac{2 \times (37,689.5956/148)}{75}} = 2.6059$			
t values for D.F. 148	t .05 = 1.979		t .01 = 2.616	

Estimation of difference

$c^Bs/cs^+ - c^Bs/c^Bs$ $(103.4356 - 95.7147)/2.6059 = 2.943$
$c^Bs/cs^+ - cs^+/cs^+$ $(103.4356 - 94.4204)/2.6059 = 3.459$
$c^Bs/c^Bs - cs^+/cs^+$ $(95.7147 - 94.4204)/2.6059 = .497$

Growth Rate

The growth rates of the three genotypes cs^+/cs^+, c^Bs/c^Bs and c^Bs/cs^+ were examined. The specimens were the progeny of the mating of BS parents reared in the laboratory of the National Institute of Genetics. Altogether 1028 young snails with the shell diameter 3–6 mm were used. These had been derived from 75 cultures, each of which was the progeny of the same parent pair and raised in the same vessel. The shell diameter of each individual was measured under a microscope, with an ocular micrometer of which one unit corresponded with .05 mm. The results obtained were analyzed by the statistical method for "randomized block" experiments (Fisher, 1951). As presented in table 2, there is a significant difference in average size of one-per cent level between the double heterozygote c^Bs/cs^+ and either homozygote cs^+/cs^+ or c^Bs/c^Bs, whereas the difference between the two homozygotes is not significant. This finding indicates that the growth rate up to this stage had been somewhat different according to genotype, and that the double heterozygote significantly surpassed either homozygote in this respect.

Susceptibility to Low Temperature

To determine whether or not there was any difference in susceptibility to adverse environmental conditions among the genotypes, various types of preliminary experiments were performed. Of these, the one concerned with susceptibility to low temperature yielded, apparently, the most significant result, so further experiments along this line were carried out. Certain preliminary surveys had made it apparent that $-5.5°$ C. was approximately the most suitable low temperature for experimentation. This is near the lowest temperature encountered in winter in the middle part of Japan. It had been found that the lower temperature $-7°$ C., lasting for an hour or 2, killed nearly all adult snails.

From 15 to 36, as a rule 25, young

TABLE 3. *Experiments on susceptibility to low temperature −5.5° C. of young individuals 3.5–5 mm in shell diameter*

Experiment		1			2			3			4			5		
Genotype		cs^+/cs^+	c^Bs/c^Bs	cs^+/c^Bs	cs^+/cs^+	c^Bs/c^Bs	cs^+/c^Bs	cs^+/cs^+	c^Bs/c^Bs	cs^+/c^Bs	cs^+/cs^+	c^Bs/c^Bs	cs^+/c^Bs	cs^+/cs^+	c^Bs/c^Bs	cs^+/c^Bs
Number																
Initial		26	15	36	25	25	25	25	25	25	23	25	24	25	25	25
After treatment	1	26	12	29	23	3	17	25	16	24	23	20	19	25	24	25
	2	22	8	28	15	0	13	25	10	23	23	19	18	23	23	25
	3	19	8	23	13	0	13	23	8	20	18	13	17	21	23	25
	4	17	7	20	9	0	13	21	7	19	14	10	14	20	18	24
	5	16	6	19	6	0	13	16	5	17	8	4	12	19	16	22
	6	16	6	18	5	0	11	16	3	14	6	2	2	19	12	19
	7	16	6	18	5	0	11	13	1	10	4	0	0	18	9	17
	8	12	2	16	2	0	5	11	1	8				17	7	14
	9	12	2	16	1	0	3	9	0	4				13	4	11
	10	12	2	14	0	0	1	7	0	2				10	1	8
	11	10	0	9				1	0	0				6	0	3
	12	5	0	0										2	0	0

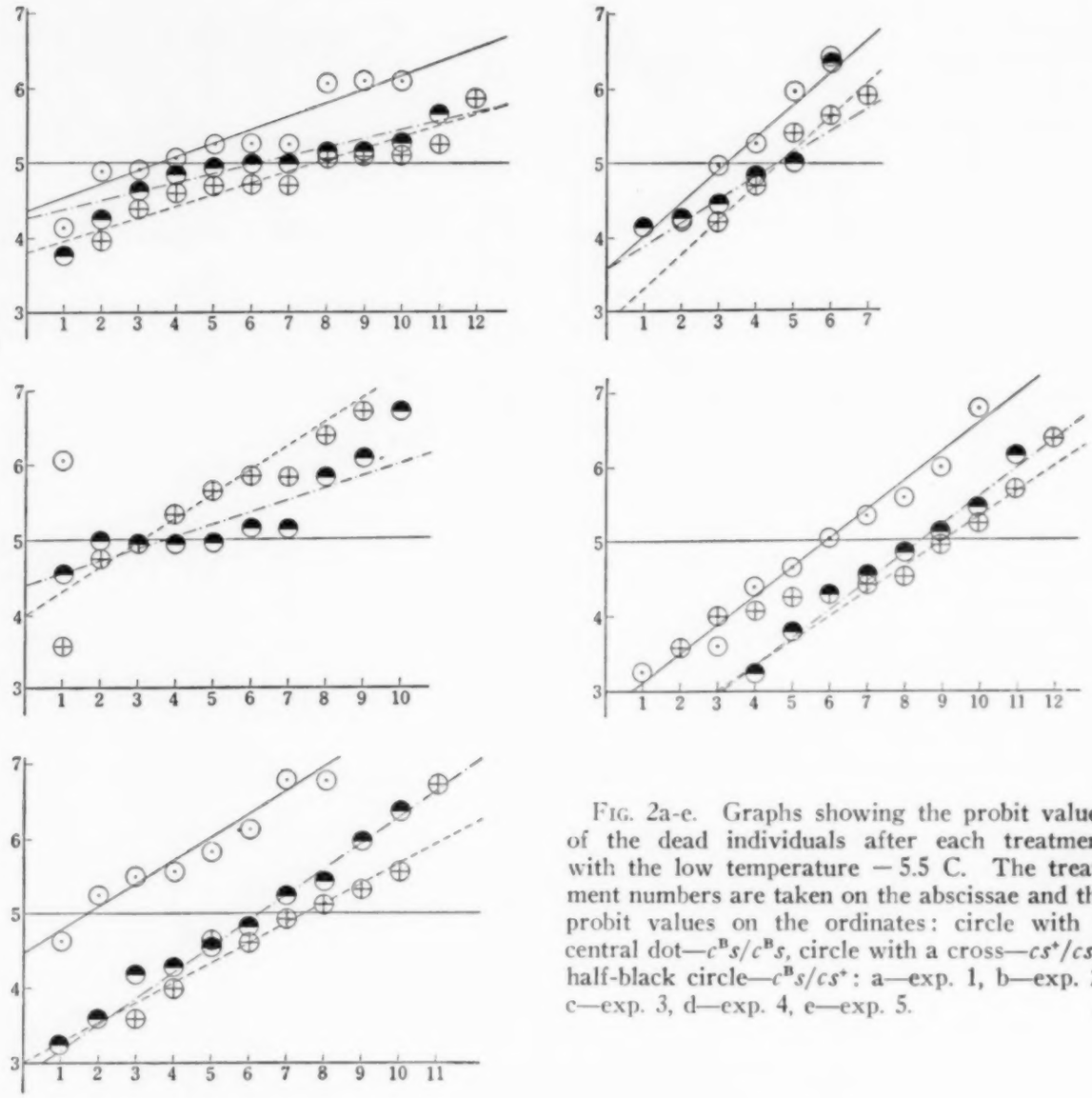

FIG. 2a-e. Graphs showing the probit values of the dead individuals after each treatment with the low temperature − 5.5 C. The treatment numbers are taken on the abscissae and the probit values on the ordinates: circle with a central dot—$c^{B}s/c^{B}s$, circle with a cross—cs^{+}/cs^{+}, half-black circle—$c^{B}s/cs^{+}$: a—exp. 1, b—exp. 2, c—exp. 3, d—exp. 4, e—exp. 5.

snails of 3.5 − 5 mm shell diameter, and of the genotypes $c^{B}s/c^{B}s$, cs^{+}/cs^{+} or $c^{B}s/cs^{+}$, were placed in a Petri dish. These snails were the same ones that had been used for the examination of growth rate. The materials in the three dishes, each of which contained one of these genotypes, made a set, and were subjected to the same treatment at the same time. They were kept in a low-temperature room regulated at − 5.5° C. for 1.5 hours. They were then taken out of the room, and left for a short while in room temperature about 17° C. Each snail was examined, whether it was still alive or not. Live and dead individuals were separated by testing by means of the prick of a needle, repeated 3 or 4 times at intervals. The surviving snails were then subjected to the next treatment by low temperature of the same grade. The interval between the consecutive treatments was about 1 hour, but it could not be made strictly uniform.

Five series of experiments were thus conducted, with the rather uniform results (table 3). These were analyzed according to Bliss's method (Bliss, 1937; Fisher, 1951, Fisher and Yates, 1949) which had been designed for judging the effect of insecticides. The treatment number in the present experiments represented the time of killing in Bliss's examples. Probit values were obtained for the pro-

portion of dead individuals after each treatment against the initial number. These values were plotted in a graphic form. It has been found that these probit values, rather than their log values, are arranged nearly on a straight line (fig. 2). Based on this graph, the distance of the M (median mortality point) from the base ($\bar{x}$) and its standard deviation (S) were found, and the variance of $\bar{x}$ and that of S were obtained by the following formulae:

$$V(\bar{x}) = \frac{S^2}{N},$$

$$V(S) = \frac{S^2}{2N - 1.5} \text{ (Bliss, 1937).}$$

These values are presented in table 4a. The difference between $\bar{x}$ values of different types in the same set was compared with the sum of the $V(\bar{x})$ values of the corresponding types, and the significance of the difference was judged. As shown in table 4b, there are significant differences between the values for BU and BS types, or between the values for BU and YS types, in all the eight comparisons except 1 in Experiment 1. In Experiment 2, 22 BU individuals out of the total 25 succumbed to the first treatment and the rest to the second. Obviously, this result is in line with the results obtained in other experiments, although it has not been subjected to the statistical treatment. The difference between the value for YS and that for BS is significant only in one (Exp. 3) of the five experiments. It seems accordingly safe to generalize that the BU type is more susceptible to the low temperature than either the BS or YS type, while no difference is found between BS and YS types.

TABLE 4a. *$\bar{x}$, S, $V(\bar{x})$, $V(S)$ values for the data presented in table 3*

Exp.	Type	$\bar{x}$	S	$V(\bar{x})$	V(S)
1	YS	7.8	6.752	1.7534	.9028
	BU	3.6	5.494	2.012	1.080
	BS	6.3	8.662	2.084	1.064
2	YS	3.18	3.080	.380	.1956
	BS	3.6	6.156	1.516	.780
3	YS	7.4	3.762	.566	.292
	BU	1.7	3.080	.380	.1956
	BS	6.3	2.856	.328	.168
4	YS	4.65	2.072	.187	.096
	BU	3.2	2.222	.198	.102
	BS	4.62	3.328	.46	.238
5	YS	9.	3.080	.380	.1956
	BU	6.	2.654	.280	.144
	BS	8.5	2.606	.272	.140

TABLE 4b. *Data to examine the significance of the differences between $\bar{x}$ values for different types*

Exp.	Types	$\bar{x}$ dif.	$V(\bar{x}) + V(\bar{x}^1)$	Significance
1	YS–BU	4.2	3.765	+
	YS–BS	1.5	3.837	–
	BU–BS	2.7	4.096	–
2	YS–BS	.42	1.896	–
3	YS–BU	5.7	.946	+
	YS–BS	1.10	.894	+
	BU–BS	4.6	.708	+
4	YS–BU	1.46	.3844	+
	YS–BS	.04	.647	–
	BU–BS	1.42	.658	+
5	YS–BU	3.0	.66	+
	YS–BS	.50	.652	–
	BU–BS	2.50	.552	+

These data have also been examined by making comparisons with respect to V (X) values at several points other than M by means of the formula:

$$V(X) = V(\bar{x}) + (Y - 5)^2 V(S),$$

where V(X) denotes the variance at the given point, $V(\bar{x})$ the variance of $\bar{x}$, V(S) the variance of the standard deviation and Y the "provisional probit." The results obtained indicate significant differences at the 90 per cent mortality point in the following comparisons:

Exp. 2	between YS and BS
Exp. 3	between YS and BU
Exp. 3	between YS and BS
Exp. 3	between BU and BS
Exp. 4	between YS and BU
Exp. 4	between BU and BS

TABLE 5. *Composition of population samples obtained from localities in Japan and Formosa. 100 times the values of p, q and r are shown in respective columns*

No.	Locality	YU	YB	BU	BS	N	p	q	r	p+q+r	D	σD
1	Hunakosi	98	38	0	0	136	15.15	0	84.85	100.00	0	—
2	Kohase	91	1	4	0	96	.53	2.11	97.36	100.00	0	—
3	Yamagata	69	0	52	0	121	0	24.49	75.51	100.00	0	—
4	Aikawa	252	0	0	0	252	0	0	100.00	100.00	0	—
5	Akatomari	153	0	0	0	153	0	0	100.00	100.00	0	—
6	Niigata	2334	175	135	7	2651	3.50	2.72	93.83	100.05	−.05	.044
7	Nanao-1	424	0	170	0	594	0	15.52	84.48	100.00	0	—
8	Nanao-2	323	0	36	0	359	0	5.15	94.85	100.00	0	—
9	Nanao-3	220	0	75	0	295	0	13.64	86.36	100.00	0	—
10	Notozima	42	0	9	0	51	0	9.25	90.75	100.00	0	—
11	Yokosuka	137	70	12	0	219	17.51	2.78	79.09	99.38	.62	.37
12	Misima	164	93	0	0	257	20.11	0	79.89	100.00	0	—
13	Hatizyô	141	0	3	0	144	0	1.00	99.00	100.00	0	—
14	Titizima	1435	0	0	0	1435	0	0	100.00	100.00	0	—
15	Sizuoka	1034	7	0	0	1041	.33	0	99.67	100.00	0	—
16	Simada	168	0	0	0	168	0	0	100.00	100.00	0	—
17	Hamamatu	202	66	0	0	268	13.18	0	86.82	100.00	0	—
18	Toyohasi	29	0	0	0	29	0	0	100.00	100.00	0	—
19	Nagoya-1	948	0	0	0	948	0	0	100.00	100.00	0	—
20	Nagoya-2	1080	0	0	0	1080	0	0	100.00	100.00	0	—
21	Gihu	54	0	0	0	54	0	0	100.00	100.00	0	—
22	Toba	210	3	0	0	213	.70	0	99.30	100.00	0	—
23	Sirakawa-1	127	19	0	0	146	6.73	0	93.27	100.00	0	—
24	Sirakawa-2	363	46	0	0	409	5.79	0	94.21	100.00	0	—
25	Sirakawa-3											
a	(Oct. '47)	497	22	22	0	541	2.02	2.02	95.86	99.90	.10	.062
b	(May '48)	486	19	20	0	525	1.83	1.92	96.21	99.96	.04	.058
c	(Oct. '48)	439	20	20	0	479	2.11	2.11	95.73	99.95	.05	.068
d	(Jun. '49)	239	10	14	1	264	2.10	2.88	95.15	100.13	−.13	.109
e	(Oct. '49)	186	11	12	0	209	2.67	2.91	94.34	99.92	.08	.14
f	(May '50)	187	6	6	0	199	1.52	1.52	96.91	99.95	.05	.078
26	Sirakawa-4											
a	(May '48)	347	73	20	7	447	9.39	3.07	88.11	100.57	−.57	.188
b	(May '50)	347	67	47	3	464	7.85	5.55	86.48	99.88	.12	.23
27	Sirakawa-5											
a	(May '48)	1097	0	6	0	1103	0	.27	99.73	100.00	0	—
b	(Jun. '48)	107	0	1	0	108	0	.46	99.54	100.00	0	—
c	(July '49)	334	0	0	0	334	0	0	100.00	100.00	0	—
d	(May '50)	175	0	1	0	176	0	.34	99.66	100.00	0	—
28	Sirakawa-6	83	0	13	0	96	0	7.02	92.98	100.00	0	—
29	Zyôdozi-7											
a	(May '48)	487	24	15	1	527	2.40	1.53	96.13	100.06	−.06	.059
b	(Jun. '49)	409	16	13	0	438	1.85	1.50	96.63	99.98	.02	.059
30	Okazaki-8											
a	(May '48)	521	74	0	0	595	6.43	0	93.57	100.00	0	—
b	(Sept. '49)	320	59	0	0	379	8.11	0	91.89	100.00	0	—
31	Tanaka-9											
a	(Jun. '48)	813	12	191	0	1016	.59	9.89	89.45	99.93	.07	.054
b	(Sept. '49)	529	6	83	0	618	.50	6.96	92.52	99.98	.02	.055
32	Simogamo-10	801	18	159	0	979	.97	8.52	90.45	99.94	.06	.068
33	Simogamo-11	233	13	43	0	289	2.28	7.74	89.78	99.80	.2	.18
34	Yosida-12											
a	(Nov. '47)	140	6	26	2	174	2.33	8.40	89.70	100.43	−.43	.25
b	(May '48)	952	53	225	3	1233	2.29	9.72	87.87	99.88	.12	.098
c	(May '50)	129	13	16	0	158	4.24	5.24	90.33	99.81	.19	.28
35	Yosida-13	216	15	41	1	273	2.97	8.02	88.95	99.94	.06	.22

TABLE 5—*Continued*

No.	Locality	YU	YB	BU	BS	N	p	q	r	p+q+r	D	σD
36	Yosida-14	702	78	21	0	801	5.00	1.31	93.62	99.93	.07	.065
37	Yosida-15	577	13	58	0	648	1.01	4.58	94.36	99.95	.05	.06
38	Nakagyô-16											
a	(July '46)	313	169	1	0	483	19.38	.10	80.50	99.98	.02	.05
b	(June '48)	149	93	7	1	250	21.01	1.61	77.23	99.85	.15	.29
39	Nisinokyô	343	79	25	3	450	9.57	3.16	87.30	100.03	−.03	.19
40	Komatubara	126	0	0	0	126	0	0	100.00	100.00	0	—
41	Takatuki	335	12	179	3	529	1.42	19.01	79.58	100.01	.01	.18
42	Ibaraki	1613	0	49	0	1662	0	1.49	98.51	100.00	0	—
43	Miyakozima	544	141	78	16	779	10.65	6.23	83.56	100.44	−.44	.23
44	Tennôzi	3835	64	2629	21	6549	.65	22.84	76.52	100.01	−.01	.04
45	Hamadera	477	38	37	11	563	4.45	4.36	92.04	100.85	−.85	.14
46	Hakotukuri	290	80	37	6	413	11.03	5.35	83.80	100.18	−.18	.29
47	Arita	350	4	0	0	354	.57	0	99.43	100.00	0	—
48	Yukawa-1	137	71	83	25	316	16.56	18.87	65.84	101.27	−1.27	.80
49	Yukawa-2	118	59	83	16	276	14.67	19.92	65.38	99.99	.01	.28
50	Yukawa-3	147	60	74	14	295	13.45	16.24	70.59	100.28	−.28	.70
51	Kusimoto-1	466	262	0	0	728	20.00	0	80.00	100.00	0	—
52	Kusimoto-2	1380	306	0	0	1686	9.53	0	90.47	100.00	0	—
53	Yasuura	1228	3	65	0	1296	.12	2.54	97.33	99.99	.01	.012
54	Takamatu	160	19	26	2	207	5.21	7.00	87.91	100.12	−.12	.31
55	Tambara-1	2482	471	0	0	2953	8.32	0	91.68	100.00	0	—
56	Tambara-2	790	65	0	0	855	2.20	0	97.80	100.00	0	—
57	Tambara-3	2120	995	0	0	3115	17.50	0	82.50	100.00	0	—
58	Niukawa	2713	44	46	0	2803	.78	.83	98.38	99.99	.01	.01
59	Komatu	390	150	0	0	540	15.03	0	84.97	100.00	0	—
60	Hôzyuzi	1102	0	0	0	1102	0	0	100.00	100.00	0	—
61	Yosii	616	47	0	0	663	3.61	0	96.39	100.00	0	—
62	Kusukawa	211	0	0	0	211	0	0	100.00	100.00	0	—
63	Matuyama	107	40	3	0	150	14.95	1.01	84.46	100.42	−.42	.25
64	Kôti-1	94	138	6	2	240	35.42	1.16	62.61	99.69	.31	.44
65	Kôti-2	149	127	4	6	286	26.86	1.77	72.17	100.80	−.80	.34
66	Kôti-3	175	73	29	5	282	14.94	6.21	78.78	99.93	.07	.46
67	Kôti-4	216	7	8	1	232	1.74	1.96	96.49	100.19	−.19	1.05
68	Kôti-5	37	5	12	0	54	4.74	11.81	82.77	99.32	.68	.78
69	Kôti-6	295	46	12	1	354	6.88	1.85	91.29	100.02	−.02	.14
70	Kôti-7	220	15	7	0	242	3.15	1.46	95.35	99.96	.04	.1
71	Kôti-8	593	143	3	0	739	10.19	.15	89.58	99.92	.08	.034
72	Susaki	53	42	0	0	95	25.29	0	74.71	100.00	0	—
73	Kawauti	48	0	0	0	48	0	0	100.00	100.00	0	—
74	Nagasaki-1	16	5	67	3	91	4.50	51.97	41.93	98.40	1.60	1.67
75	Nagasaki-2	110	13	295	13	431	3.07	46.59	50.52	100.18	−.18	.56
76	Nagasaki-3	165	26	134	9	334	5.39	24.38	70.25	100.02	−.02	.523
77	Nagasaki-4	117	70	208	54	449	14.92	35.47	51.04	101.43	−1.43	1.033
78	Nagasaki-5	278	280	482	145	1185	19.92	31.38	48.44	99.74	.26	.69
79	Nagasaki-6	213	229	466	146	1054	19.74	35.24	44.96	99.94	.06	.80
80	Nagasaki-7	16	35	46	21	118	27.50	34.26	36.82	98.58	1.42	2.90
81	Nagasaki-8	131	170	258	99	658	23.10	32.36	44.62	100.08	−.08	1.04
82	Nagasaki-9	150	124	231	72	577	18.75	31.10	50.98	100.83	−.83	.96
83	Amakusa	36	55	38	27	156	31.13	23.62	48.04	102.79	−2.79	2.112
84	Akuné	43	6	0	0	49	6.49	0	93.51	100.00	0	—
85	Taipei	340	25	25	1	391	3.38	3.38	93.25	100.01	−.01	.126
86	Karenkô	8	110	0	0	118	73.96	0	26.04	100.00	0	—

Thus, the BU type (c^Bs/c^Bs) shows higher susceptibility to the low temperature at the 90-per cent mortality point than either the YS or BS type in four of the eight comparisons. A similar difference appears to be present between YS

TABLE 6. *Chi-square tests of the differences between the observed and expected numbers of the four types in the samples which show D values exceeding twice σD*

Sample		YU	YS	BU	BS	Total	D.F.	P
26a	Observed	347	73	20	7	447		
	Expected	342.85	77.20	24.38	2.56	446.99		
	Difference	+4.15	−4.20	−4.38	+4.44			
	Dif.²/Exp.	.05	.23	.79	7.7	$\chi^2=8.77$	1	<.01
45	Observed	477	38	37	11	563		
	Expected	468.48	46.72	45.64	2.17	563.01		
	Difference	+8.52	−8.72	−8.64	+8.83			
	Dif.²/Exp.	.16	1.63	1.64	35.93	$\chi^2=39.35$	1	<.001
65	Observed	149	127	4	6	286		
	Expected	146.14	129.86	7.30	2.70	286		
	Difference	+2.86	−2.86	−3.30	+3.30			
	Dif.²/Exp.	.06	.06	1.49	4.03	$\chi^2=5.64$	1	.01–.02

and BS in Experiments 2 and 3. The data in these two experiments, however, are contradictory to each other, the V(X) value is higher for BS than for YS in Experiment 2, while the reverse is true in Experiment 3.

It is to be noted that there were some differences in sizes among the materials used in these experiments. The BS snails were on the average larger than either BU or YS snails. The significant difference in susceptibility to low temperature between BS and BU snails might be in part due to this difference in growth stage. This, however, can not be true of the equally significant difference between YS and BU snails which were of about the same average size.

POPULATION SAMPLES

Population samples of *Bradybaena similaris* were obtained from 84 localities in Japan and 2 localities in Formosa. Some of these localities supplied us with samples on more than one occasion, so there were altogether 103 samples of natural population available for our study (table 5). The area of each locality was small, mostly a few acres. Some were mere strips of land about 10 meters long and 2 meters broad. A few localities, which yielded more than 1000 specimens in a single collection, were larger. Even these larger areas are designated as one locality, when they were continuous stretches of land which seemed to allow free range of the snails. It is somewhat doubtful whether this rule holds for the samples supplied by our collectors. Possibly specimens obtained from more than one isolated population were mixed together and designated as derived from the same population. The effect of such mixture, if any, is not apparent as far as our analyses go.

The analyses were made according to the following formulae:

$$r = \sqrt{\overline{YU}},$$

$$p = 1 - \sqrt{(\overline{YU} + \overline{BU})},$$

$$q = 1 - \sqrt{(\overline{YU} + \overline{YS})},$$

where r stands for the incidence of the recessive gene for yellow-unbanded, p for that of the dominant gene for banding and q for that of another dominant gene for brown color. The sum of p, q and r should be equal to unity, if these color and banding types are due to genes which are essentially triple-allelic. The standard error σD has been found by the formula:

$$\sigma D = \frac{1}{\sqrt{N}} \sqrt{\frac{pq}{2(1-p)(1-q)}}.$$

If D, the difference between the sum of p, q, r and the unity, exceeds twice σD, D is considered significant (Bernstein, 1930a).

Composition of the Samples

Of the 103 samples, 55 have positive values for all p, q and r, and the sum of these values in each sample does not differ significantly from unity except in three samples. This fact clearly sustains the validity of the above genetic interpretation of the color types. It also suggests that the breeding in nature has been panmictic as regards the color types, and that there has been no selective elimination among them.

The 3 exceptional samples, Nos. 26a, 45 and 65, have been more crucially examined in the following way: The values of p, q and r are adjusted by the formulae:

$$p' = p(1 + D/2),$$
$$q' = q(1 + D/2),$$
$$r' = (r + D/2)(1 + D/2)$$

(Bernstein, 1930b).

By use of these adjusted values, expected numbers of different types are obtained. The numbers were compared with the corresponding observed numbers, and the significance of the difference was examined by chi-square test (Stevens, 1938). The results are presented in table 6.

In all these samples P is lower than .02. It is common to these samples that the observed values of the double heterozygote and the recessive exceed the corresponding expected values, while the observed values of both the single dominants are smaller than the corresponding expected values. This fact suggests that the double heterozygote, as well as the recessive, among these samples, has had some selective advantage to the single dominants. A similar tendency has been recognized in natural populations of the polymorphic butterfly *Neozephyrus taxila* (Komai, 1953).

Geographic Variation Among the Samples

The recessive YU type forms the majority of all populations in Japan. In terms of gene frequency, r usually makes more than 80 per cent, and the rest is divided between p and q. The value of r is less than 50 per cent only in samples from a few localities in Kyûsyû.

There is a general tendency for the populations inhabiting the same district to have similar genic compositions, although there is considerable geographic variation within Japan. Proceeding from north to south, we find that the samples from Yamagata (no. 3) and Noto (nos. 7–10) on the Japan Sea coast include fairly large proportions of the brown type, and are lacking in the banded type. The samples from the two localities of Sado Island (nos. 4, 5) consist entirely of the recessive type YU. The samples from the three localities on the Pacific coast of Middle Japan, Yokosuka (no. 11), Misima (no. 12) and Hamamatu (no. 17), on the contrary, include a fairly large proportion of the YS type, while the samples from the latter two localities are lacking in the BU type. The samples of Simada (no. 16), Toyohasi (no. 18), Nagoya (nos. 19, 20) and Gihu (no. 21), the more westward cities in Middle Japan, consist entirely of the YU type. The sample of Toba (no. 22), located at the base of Kii Peninsula on the east coast, comprises the banded form in a small proportion; this is also the case with the samples of Arita (no. 47), located on the other side of the base of the peninsula. The samples of Yukawa (nos. 48–50), farther south on the west coast, include both the banded and brown types in fairly high proportions. The brown type is missing in the samples of Kusimoto (nos. 51, 52) at the southernmost point of Kii Peninsula. The samples of Osaka and the vicinity (nos. 41–46) are rather variable in composition. In general, all the 4 types are represented with various p and q values. This is true of the majority of the samples of various localities in Kyoto which will be mentioned later.

All the samples from the north coast of Sikoku (nos. 54–62) share the feature that q has a very low value, ranging from zero to one, and p varies rather widely

from zero to 20, except for the samples of Takamatu (no. 54), which resembles the Osaka and Kyoto samples. The majority of the samples from various places in the city of Kôti and vicinity (nos. 64–73), on the south coast of Sikoku, resemble the samples from the north coast of Sikoku mentioned above, but they show rather high variability among themselves in the fraction of both the banded and brown individuals. The samples from Nagasaki (nos. 74–82) and Amakusa (no. 83) on the west coast of Kyûsyû are characterized by the high incidence of c^B gene, the value of q amounting to 30–50 per cent, the highest among all the samples we have.

We have also samples obtained from the smaller islands, Hatzyô (no. 13) and Titizima of the Bonin Group (no. 14). Both the samples consist almost exclusively of the YU type, with a few BU individuals mixed in the Hatizyô sample.

Geographic Variation Among the Samples from the Localities Within the Same City or District

The general tendency that adjacent localities are inhabited by populations similar in composition may be recognized in samples that come from different quarters within the same city or district also. For instance, all the samples from various quarters in Kyoto share the characteristic that both the YS and BU types are represented in low proportions. These proportions, however, vary from population to population, over a very limited range. Thus, in the samples from Sirakawa (nos. 23–28) and Zyôdozi (no. 29) in the northeast corner of the city, r occupies from 86 to 100 per cent, and the rest is divided among p and q in various proportions. Most of the samples from Tanaka (no. 31), Simogamo (nos. 32, 33) and Yosida (nos. 34–37), in the east quarter of the city, differ from the Sirakawa-Zyôdozi samples in having larger q, amounting nearly to 10 per cent. The samples from Nakagyô (no. 38) and Nisinokyô (no. 39), located in the western quarter, have relatively large p ranging from nearly 10 to 21 per cent.

Differences Among the Samples from Adjacent Localities

Although, as a general rule, samples from adjacent localities have similar compositions, exceptions are not rare. For instance, Sirakawa-2, 3 and 4 in Kyoto are localities that lie within a range of 100 meters. Still, sample no. 24 from Sirakawa-2 includes more than 10 per cent YS, while lacking entirely in BU; no. 25 from Sirakawa-3 has both YS and BU types though in small proportions; no. 26 from Sirakawa-4 has many YS mixed with a few BU. Next, Sirakawa-5 is a short strip of land hardly 30 meters long which forms a part of the court yard of the quadrangle building of the College of Agriculture of Kyoto University. It is inhabited by a characteristic population (nos. 27 a-d), which consist almost exclusively of YU individuals, mixed with a very few BU with q lower than 0.5 per cent. All the samples from the north coast of Sikoku (nos. 55–62) show variation of a rather wide range within a limited area of habitat; especially the value of p varies from 2 to 35 per cent.

It is also to be noted that, in spite of the general tendency that adjacent colonies are of similar composition, there is nothing that suggests the formation of a cline in the range of this snail in contrast to conditions such as found in the distribution of the variable lady-beetle *Harmonia* through the Japanese main island chain (Komai, Chino and Hosino, 1950).

Coincidental Resemblance of the Samples from Distant Localities

While samples from adjacent localities, as shown above, may be occasionally of distinct compositions, samples from distant localities may be of very similar compositions. The following is an example:

Sample no.	Locality	p	q	r
1	Hunakosi	15.15	0.00	84.85
17	Hamamatu	13.18	0.00	86.82
59	Komatu	15.03	0.00	84.97.

High incidence of c^B gene may be found in populations of such distant colonies as of Nanao (nos. 7, 8), Takatuki (no. 41), Tennôzi (no. 44), Yukawa (nos. 48–50), Nagasaki (nos. 74–82) and Amakusa (no. 83). Monomorphic populations with r = 100 occur in localities that are distant from one another and very diverse in physical conditions, such as Sado Island (nos. 4, 5), Nagoya-Gihu district (nos. 16, 18–21), Ehimé (no. 60), Titizima (no. 14), and a few others. These resemblances of distant colonies are obviously due to mere coincidence; they are never to be attributed to similarity of habitat, or to common descent of these colonies.

Composition of the Samples from the Same Locality Obtained on Different Occasions

Some localities have supplied us with samples on more than one occasion, in different seasons or in different years. For instance, Sirakawa-3 is Komai's vegetable garden where samples (nos. 25 a-f) were obtained on 6 occasions from October 1947 through May 1950. The analysis of these samples shows that the composition of the colony has remained unchanged during this period. This finding, though meager, suggests that the breeding was pan-mictic, and there were no differential survivals according to the color types.

Incidentally, it may be added, that the snails of this species show rather considerable variation in several other characters, such as, the shape of the shell which may be conical or flattened, the breadth of the band, the tone of the shell color in both yellow and brown, and in the marking of the soft body. These variations, at least in most cases, seem to be genetically controlled.

Discussion

The polymorphism found in *Bradybaena similaris* undoubtedly belongs to the "balanced" type (Fisher, 1927, 1930, 1931; Ford, 1940, 1953; Sheppard, 1953a, b; Huxley, 1948; Dobzhansky, 1951). There are four distinct color and banding types which are maintained in a stable state. These types are apparently governed by a set of para-allelic genes which behave actually as triple-allelic genes. Although no conclusive evidence is available for either of the possibilities, para-allelism or real triple-allelism, the former is more plausible than the latter. First, the phenotypical effect of these genes is qualitatively different instead of quantitatively so, i.e. one of the genes changes the ground color, and the other produces the band. Second, the combined effect of both the genes is manifested in a mosaic form (Komai, 1950). According to this view, the gene symbols of the four types become: cs/cs (YU); c^Bs/c^Bs, c^Bs/cs (BU); cs^+/cs^+, cs^+/cs (YS) and c^Bs/cs^+ (BS). It has been demonstrated that the double heterozygote c^Bs/cs^+ surpasses both the single dominant homozygotes c^Bs/c^Bs and cs^+/cs^+ in growth rate, and also that the c^Bs/cs^+ and cs^+/cs^+ surpass c^Bs/c^Bs in the power of resistance to low temperature. It is rather strange that these differences in physiological properties among the genotypes are not manifested in our breeding data. This might be due to the fact that the laboratory populations were reared under rather favorable conditions, and that they were never subjected to such temperature as low as $-5.5°$ C. Furthermore, the effect of selection by predatory animals was largely excluded. In any event, this finding is in contrast to the breeding data presented by some previous authors on polymorphic animals where the heterozygotes significantly exceeded the numerical ratio expected by Hardy-Weiberg's law, for example, in *Apotettix texanus* (Fisher, 1930), *Paratettix texanus* (Fisher, 1939), *Drosophila polymorpha* (da Cunha, 1949), *Ephestia küniella* (Caspari, 1950) and *Drosophila pseudoobscura* (Dobzhansky, 1947, 1950; Dobzhansky and Levene, 1948).

The difference among the genotypes with respect to the physiological properties mentioned above also is not manifested by the samples of natural populations de-

rived from various localities except in 3 samples. In these 3 it has been shown that the double heterozygote and recessive have selective advantage to single dominants. The selective advantage of heterozygote to homozygotes has been demonstrated in natural populations of *Paratettix texanus* (Fisher, 1939), *Drosophila pseudoobscura* (Dobzhansky and Levene, 1948; Dobzhansky, 1951) and of the butterfly *Neozephyrus taxila* (Komai, 1953).

The polymorphism found in *Bradybaena similaris* represents a type which might be called "compound" polymorphism, in contrast to the "simple" type of polymorphism dealt with by many previous authors. We have here 4 phenotypes and 6 genotypes involving 2 loci, whereas the previous authors had polymorphisms consisting of 3 genotypes and 2 or 3 phenotypes involving a single locus. We have found differences in physiological properties among 3 of the 6 genotypes, while the remaining 3 genotypes are yet to be examined. It is possible that the relative inferiority of the dominant homozygotes among the natural populations in these physiological properties is compensated by the relative superiority of the heterozygotes which constitute the majority of the corresponding phenotype. If so, the effect of such physiological differences will be masked.

In spite of this difference between the polymorphism of this snail and that of other animals, it is very likely that the polymorphism of the former is maintained by an antagonism of selective advantage and disadvantage of the constituent genotypes, as in other cases. It is conceivable from an *a priori* ground that both the brown color and banding confers some advantage to the animal possessing them, since the gene responsible for either character shows complete dominance in the heterozygote. It remains, however, to be shown in what way this color or marking is beneficial to its bearer. Either character presumably would become disadvantageous, in some way, if the genes became homozygous, for otherwise they would replace the recessive gene completely. As a matter of fact, the physiological inferiority of the homozygotes has been disclosed in regard to growth rate and susceptibility to low temperature. The basic mechanism of the maintenance of this compound polymorphism seems to be the same as in the polymorphism of other animals, although much still remains to be investigated.

There is another problem which concerns with the cause of differentiation of local populations of *Bradybaena similaris*. Discussion concerning *Cepaea* has been carried on recently on this problem between Cain and Sheppard on the one hand and Lamotte on the other. The former authors hold the view that visual selection by thrushes plays a decisive part in this differentiation, while the latter author attributes it to genetic drift, as well as to gene flow. For further details the reader is referred to the recent papers by Cain and Sheppard, 1954 and by Lamotte, 1951, 1952. Our observations on *Bradybaena* have shown that the populations occurring in adjacent localities are generally similar in composition, but they are occasionally much different; also, that some populations that inhabit distant localities may show striking coincidence. The geographical differentiation of populations, on the whole, seems to be related in no way to difference in climate, vegetation or soil character. No regular gradient, such as observed in the distribution of the ladybeetle *Harmonia,* which is obviously correlated with the climatic gradient, occurs. The geographical variations in the composition of the populations of this snail appear fortuitous, and not correlated with differences in environmental conditions. This is exactly what has been observed by many previous authors on the distribution of *Cepaea* (Schilder, 1923, 1925, 1948; Aubertin, 1927; Rensch, 1933; Diver, 1932, 1939, 1940; also Robson, 1928; Robson and Richards, 1936; Mayr, 1946; Huxley, 1942 and Lamotte, 1951a, b, 1952). Furthermore, different types in each colony of the present species are

represented in a numerical ratio expected on the postulate that there is no differential elimination of any special type. Some colonies have been observed to have remained unchanged through several generations. This is in line with the conclusion by Diver (1929) that certain local populations of *Cepaea* had been stable since the Pleistone age. We have no positive evidence that the physiological difference among some genotypes has ever taken part in the differentiation of the local populations. Even were this the case, the effect should have been compensated by some antagonistic effect which has not been demonstrated as yet. The visual selection by predatory birds seems to be virtually out of question.

As stated above, the distribution of this snail has undoubtedly depended a great deal on human agency. When it was introduced into a new habitat, obviously only a few individuals were brought in from a neighboring locality, rather than a large number from a distant locality. It is also probable that this snail occasionally migrated into a new habitat. Even then, there was evidently small chance that it migrated from a distant locality in large groups. These circumstances probably account for the general tendency that adjacent populations have similar compositions. It is very likely that these immigrants were often inadequate representatives of the parent colony, and they became the ancestors of a new colony much different in composition from the parent colony. This small number and misrepresentation of immigrants which may be designated as "bottle-neck" effect, seems to be the most important cause of the differentiation of natural populations of this snail. Besides, there must have been the genetic drift due to the small size of breeding population. Schilder (1948, 1950) has ventured a similar opinion about the distribution of *Cepaea* spp. Lamotte (1952) puts more emphasis on the effect of genetic drift than gene flow by migration.

Lastly, we shall discuss the origin of natural polymorphism in general. This phenomenon implies the co-existence of more than one distinct types in a lasting and stable state. Since these types are usually morphological (visible) ones, we shall limit our discussion to such morphological polymorphisms. The same argument applies in principle to physiological ones such as found in blood groups. It seems that the balanced mechanism advocated by Fisher, Ford and others is indispensable for keeping the polymorphism in a lasting and stable state. This mechanism requires that the gene or gene arrangement controlling this phenomenon be relatively deleterious in the homozygous state and advantageous in the heterozygous state. This advantage or disadvantage is judged in comparison with that of the recessive gene, which produces the "universal recessive"—probably the "wild" type. There should be some beneficial effect due to the dominant or semidominant gene; the degree of dominance is probably correlated with the degree of benefit that is conferred.

The "compound" type of polymorphism represented by the *Bradybaena similaris* is probably governed by a set of multiple-allelic or para-allelic genes which behave according to the same principle as that given above for "simple" polymorphism. In reality, we seem to have para-allelic genes instead of multiple-allelic genes in the majority of the cases of compound polymorphism. We have presented above the evidence for this view. For further evidence it may be pointed out that various degrees of linkage relations are known for the genes responsible for the polymorphism in *Cepaea* (Sheppard, 1953; Cain and Sheppard, 1954).

The close linkage of the para-allelic genes is probably maintained by a chromosome rearrangement, especially by inversion. Should this system break down frequently by crossing over, a stable polymorphism could never be present. Probably most, if not all, cases of stable polymorphism are maintained by such chromosomal mechanisms. The findings by

Dobzhansky and his associates of the great variety of inversions in *Drosophila pseudoobscura* are highly suggestive in this connection.

The compound polymorphism, such as found in *Bradybaena similaris,* probably involves more than one rearrangement with, for instance, one chromosome carrying a mutant gene for shell color and the other chromosome carrying another mutant gene for shell banding. Since crossing over between these chromosomes, as well as between one of them and the uninverted chromosome, will be largely inhibited, a compound poly rphism will result.

Summary

1. *Bradybaena similaris* is a land snail distributed widely in coastal regions of the warm parts of Asia, as well as of Africa, Australia and in some parts of Central and South America.

2. This species is polymorphic, with four color and banding types, namely, yellow-unbanded (YU), yellow-banded (YS), brown-unbanded (BU) and brown-banded (BS). The distribution, life habit and the method of rearing of this snail are described.

3. The breeding data indicate that this polymorphism is governed by two sets of para-allelic genes which actually behave as triple-allelic genes.

4. There are some physiological differences among the genotypes. The double heterozygote BS surpasses both the homozygotes, which are included in YS and BU groups, in the rate of growth to a certain stage. The former also has a higher power of resistance to low temperature, − 5.5° C., than the homozygotes among the BU group.

5. 103 population samples from 86 localities in Japan and Formosa have been analyzed according to the genetic principle borne out by the breeding data. They conform well to this principle, as well as to the assumption that the breeding is pan-mictic, and there is no differential survival according to the color types. Only in 3 samples do the observed ratios of the 4 types differ significantly from the expected ratios, so that the BS and YU groups are too large and the YS and the BU groups are too small.

6. As a general rule, adjacent colonies have similar compositions, and distant colonies are distinct from each other. Exceptions, however, are not rare. No correlation is found between the composition of populations and the environmental conditions. No cline is apparent.

7. The polymorphism in this snail belongs to the balanced type. The differentiation of local populations seems to be primarily due to the "bottle-neck" effect on the immigrants to a new habitat. The genetic drift is apparently another important factor. The effect of natural selection seems to be of relatively minor importance.

8. The origin of balanced polymorphism in general is discussed.

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RANDOM GENETIC DRIFT IN MULTI-ALLELIC LOCUS[1]

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INTRODUCTION

In the theory that evolution is an irregularly shifting state of balance, especially that of a subtle balance between the evolutionary pressures of mutation, immigration and selection as a group and random or stochastic processes which give rise to processes of trial and error (Wright, 1950), the process of random genetic drift is one of the problems first to be clarified.

Actually the problem of the random drift has been discussed by various authors. As early as 1921, Hagedoorn and Hagedoorn pointed out that the group of organisms destined to become the parents of the next generation is usually considerably smaller than the number of individuals of their species so that some genes will be lost by chance. According to their opinion, reduction of potential variability is automatic, being independent of any sort of selection. They considered that this is the most important gain in knowledge that we owe to Mendel's work and to the biomechanical interpretation of it.

The first mathematical treatment of this so-called "Hagedoorn Effect" was carried out by Fisher (1922). It was rather unfortunate that an abbreviation of a term in his differential equation led him to the erroneous result that the rate of decrease of the variance in the population is $1/4n$ per generation, where n is the number of individuals in the population. Independently, Wright worked on this problem and obtained the correct answer of $1/2N$ for the first time, where N is the effective population number (see Wright, 1931). Using an integral equation he also arrived at the flat distribution of unfixed classes for the state of steady decay. This stimulated Fisher to re-examine his results and he found himself in entire agreement with Wright's results. Furthermore, Fisher elaborated the terminal part of the distribution for the case of steady decay by his method of a functional equation (Fisher, 1930a, b). This method was followed by Haldane (1939) to treat the more general case where the number of descendants does not necessarily follow the Poisson distribution. Until now, Fisher's method remains as an unique and powerful tool to find the exact distribution at the terminal part.

In 1945, Wright introduced the Fokker-Planck equation to solve the problem of the gene frequency distribution and applied the equation to the case of steady decay. The problem of random drift was also treated as a problem of a finite Markov chain by Malécot (1944), who got the asymptotic formula for a very large number of generations which is essentially the same as the previously known formula for steady decay. His result on the characteristic roots was later corrected by Feller (1951), who succeeded in getting all characteristic values of the matrix of transition probabilities.

In spite of all these works, the theoretical results have been largely at the level of asymptotic formulae, and no complete solution has been published until the present

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author reported the complete solution with a continuous model (Kimura, 1955), of which brief exposition will be made in the first part of this paper.

The problem of random drift due to small population number has also attracted much attention from experimental geneticists and many model experiments have been performed to verify the mathematical deductions or to demonstrate the phenomenon in the classroom. Among them, Dubinin and Romashov (1932) (cited from Dobzhansky, 1951), Bonnier (1947), Moody (1947) and House (1953) may be cited. Recently studies on this phenomenon using laboratory populations of Drosophila melanogaster have been done by several authors; namely by Merrell (1953) using several recessive sex-linked and autosomal genes, by Kerr and Wright (1954) using the gene *forked*, and by Prout (1954) using lethal genes.

In natural populations, some evidence has been found that may suggest the effectiveness of random drift in determining the genetic structure of the population. Among them Lamotte's works (1951, 1952) on *Cepaea nemoralis* and Haga and Kurabayashi's work (1954) on *Trillium kamtschaticum* may be especially interesting. The later authors found, through their ingenious application of the phenomenon known as the differential reactivity of the chromosomes, that in natural populations of this plant high homozygosity correlates with the smallness of population size and high heterozygosity with largeness.

In most of the experimental works mentioned above, however, selection was the predominating factor for determining the genetic change of the populations.

The theoretical treatment of the problem of the random genetic drift has been confined so far to the case where only a pair of alleles exists. This restriction, however, seems to be not only unnatural but also undesirable in some cases. In fact, Haga and Kurabayashi's work suggests the necessity of the extension to the multi-allelic case. The purpose of the present paper is therefore to give the general treatment of the problem in the case of a multi-allelic locus. It will be convenient, however, to give first the brief explanation on the case of two alleles and proceed from the special case to the general.

Summary of the Results Obtained in the Case of a Pair of Alleles

Consider a random mating population of N breeding individuals of diploid organism and let A and A' be a pair of alleles whose frequencies in the population are x and $1 - x$ respectively.

To simplify the discussion, we assume an idealized situation where selection, migration and mutation are absent and generations do not overlap. In this finite population, the gene frequency changes fortuitously from generation to generation due to random sampling of gametes in reproduction. This stochastic process of the change in gene frequency is adequately described by giving the transition probability $f(x;p;t)$ that the frequency of A in the t^{th} generation will be x when it started from the frequency p in the 0^{th} generation. Here x is a discrete random variable which takes values between 0 and 1 in steps of $1/2N$.

In natural populations, however, the number of breeding individuals will usually be large enough such that x can be treated as a continuous random variable with good approximation. This assumption of the continuous model simplifies the mathematical treatment tremendously. The only point we must take care of is that the process of random fixation or loss of an allele is irreversible and the probability distribution has definite discontinuities at both termini, $x = 0$ and 1. So we must essentially specify 3 probabilities denoted by $f(1;p;t)$, $f(0;p;t)$ and $\phi(x;p;t)dx$ giving respectively the probabilities that A becomes fixed in the population by the t^{th}

generation, that A is lost by the same generation and that the frequency of A lies between x and $x + dx$ $(0 < x < 1)$ in the t^{th} generation. The last expression has real meaning for x between 0 and 1 exclusive and will be used as a good approximation of $f(x;p;t)$ with dx being substituted for $1/2N$.

The first probability $f(1;p;t)$ can be obtained by using a moment formula for the distribution and only the results will be presented here:

$$f(1;p;t) = p + \sum_{i=1}^{\infty} (2i + 1)pq(-1)^i F(1-i,i+2,2,p)e^{-[i(i+1)/4N]t}, \tag{1}$$

where p is the initial frequency of A and $q = 1 - p$. F in this expression represents the hypergeometric function which is defined by

$$F(1-i,i+2,2,x) = 1 + \frac{(1-i)(i+2)}{1\cdot 2}x + \frac{(1-i)(2-i)\cdot(i+2)(i+3)}{1\cdot 2\cdot 2\cdot 3}x^2 + \cdots$$

$$(i = 1, 2, 3\cdots)$$

For $i = 1, 2, 3, 4$ etc., it is $1, 1 - 2x, 1 - 5x + 5x^2, 1 - 9x + 21x^2 - 14x^3$ etc. respectively. Putting $p = (1 - r)/2$, (1) can be expressed also by

$$f(1;p;t) = p + \sum_{i=1}^{\infty} \frac{(-1)^i}{2}\{P_{i-1}(r) - P_{i+1}(r)\}e^{-[i(i+1)/4N]t}, \tag{2}$$

where $P_n(r)$ represents Legendre polynomials: $P_0 = 1$, $P_1 = r$, $P_2 = \frac{1}{2}(3r^2 - 1)$, $P_3 = \frac{1}{2}(5r^3 - 3r)$ etc.

The second probability $f(0;p;t)$ of A being lost or A' being fixed by the t^{th} generation is obtained simply by replacing p with q, and r with $-r$ in the above expressions.

The third probability $f(x;p;t)$ is expressed by $\phi(x;p;t)dx$ under the continuous model. It can be shown that the probability density $\phi(x;p;t)$ satisfies the partial differential equation (Wright 1945, Kimura 1954);

$$\frac{\partial\phi}{\partial t} = \frac{1}{4N}\frac{\partial^2}{\partial x^2}\{x(1 - x)\phi\} \tag{3}$$

with an initial condition of

$$\phi(x;p;0) = \delta(x - p),$$

where δ represents the delta function. The solution

$$\phi(x;p;t) = \sum_{i=1}^{\infty} pqi(i + 1)(2i + 1)F(1-i,i+2,2,p)F(1-i,i+2,2,x)e^{-[i(i+1)/4N]t} \tag{4}$$

gives the probability density that A lies between x and $x + dx$ in the t^{th} generation $(0 < x < 1)$. The series is shown to be uniformly convergent for $t > 0$, and for large t the exponential terms decrease very rapidly, so we have an asymptotic formula:

$$\phi(x;p;t) \sim 6pqe^{-(1/2N)t} + 30pq(1 - 2p)(1 - 2x)e^{-(3/2N)t} + \cdots \tag{5}$$

(Kimura, 1954). It is interesting to note that Malécot's (1944) result

$$\theta_{rt}^{(n)} \sim (3/N)(r/2N)(1 - r/2N)k^n$$

is the first term of the above expansion.

Figure 1a illustrates the process of change in the distribution of unfixed classes when the initial gene frequency is 50% ($p = \frac{1}{2}$). It will be seen that after 2N generations the distribution curve becomes almost flat and still the genes are unfixed in about 50% of the cases. In fig. 1b the initial gene frequency is assumed to be 10% ($p = 0.1$). In this case it takes 4N or 5N generations before the distribution curve becomes practically flat. By that time, however, the genes are fixed in more than 90% of the cases. So the simplest asymptotic formula $\phi \sim Ce^{-(1/2N)t}$ may not be as useful as previously thought.

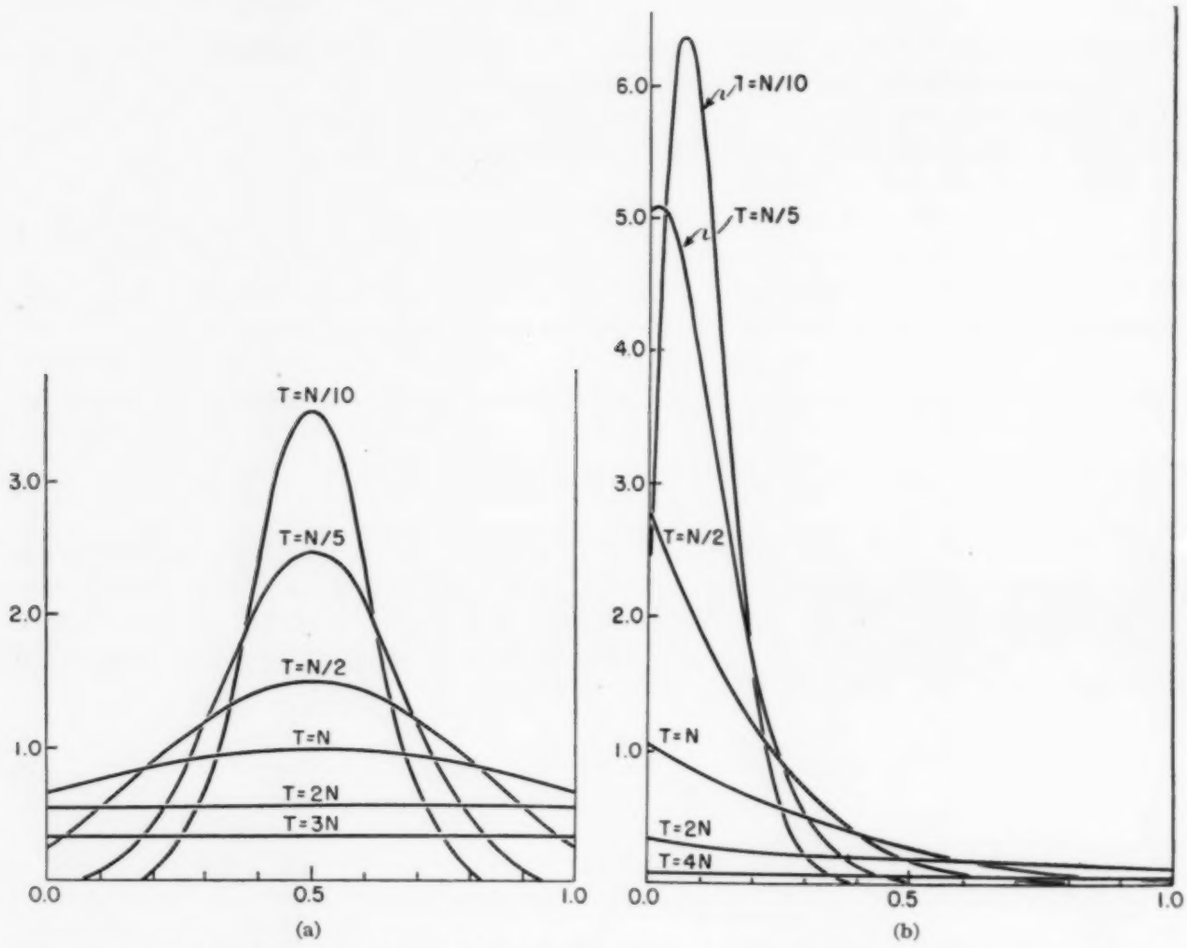

FIG. 1a and 1b. Graphs showing the process of change in the frequency distribution of unfixed classes due to random genetic drift. In this case a pair of alleles is assumed to exist. In *1a* the initial gene frequency is 50%, while in *1b* it is 10%. N is effective population size; t; number of generations elapsed, ordinates; probability densities, abscissae; the gene frequencies.

The probability that A and A' still co-exist in the population after t generations is easily calculated from the above formula (4);

$$\Omega_t = \int_0^1 \phi(x,p;t)dx = \sum_{j=0}^{\infty} \{P_{2j}(r) - P_{2j+2}(r)\}e^{-[(2j+1)(2j+2)/4N]t}. \qquad (6)$$

For $t > 0$ the series is easily seen to be convergent and as $t \to \infty$, Ω_t goes to zero, giving an asymptotic formula;

$$\Omega_t \sim 6pqe^{-(1/2N)t}. \qquad (7)$$

For $t = 0$ it can be proved that Ω_t converges to 1. Furthermore from (2) and (6)

we can verify the relation:

$$f(1;p;t) + \Omega_t + f(0;p,t) = 1.$$

The probability that a randomly chosen individual is heterozygous can be calculated from (4):

$$H_t = \int_0^1 2x(1 - x)\phi(x,p;t)dx = 2pqe^{-(1/2N)t} = H_0e^{-(1/2N)t}, \tag{8}$$

showing that the heterozygosis decreases exactly at the rate of $1/2N$ per generation. This fact was already shown by Wright (1931) by his method of path coefficients. It is also confirmed by the following calculation. Let p be the frequency of *A* in the population where the frequency of heterozygotes is 2pq. The amount of heterozygosis to be expected after one generation of random mating is

$$\begin{aligned} E\{2(p + \delta p)(q + \delta q)\} &= 2pq - 2E(\delta p)^2 \\ &= 2pq\left(1 - \frac{1}{2N}\right), \end{aligned}$$

since $\delta q = -\delta p$, $E(\delta p) = 0$ and $E(\delta p)^2 = pq/2N$.

A Note on the Effect of Population Structure

In the present paper, in order to avoid unnecessary complications, an extremely simple population structure is assumed; mating is at random and the mode of reproduction is such that N male gametes and N female gametes are drawn as a random sample from the whole population and the resulting N individuals form the next generation. Also selection, migration and mutation are assumed to be absent and generations do not overlap.

In nature, population structures are not so simple, and the concept of effective size must be introduced. The main results on this problem may be found in Wright (1931 and many of his later papers), Crow (1954), Crow and Morton (1955) and Kimura (1952). Therefore only few remarks will be made here.

1) If the population size N changes gradually from generation to generation in a deterministic way such that N_t can be represented as a continuous function of t (still $N \gg 1$), t/N in the exponential term of the previous formulas must be replaced by $\int_0^t (dt/N_t)$. For example (7) and (8) become

$$\Omega_t \sim 6pq \exp\left(-\int_0^t \frac{dt}{2N_t}\right) \tag{9}$$

and

$$H_t = 2pq \exp\left(-\int_0^t \frac{dt}{2N_t}\right) \tag{10}$$

respectively.

Thus a necessary and sufficient condition that for a growing population, H_t and Ω_t to vanish at the limit when t becomes ∞ is that the integral $\int_0^\infty (dt/N_t)$ diverges, i.e. N_t must be at most of the order of t at the limit. If the population increases

more rapidly, heterozygosis can not be eliminated entirely. This agrees with the results obtained by Malécot (1948) who used a different method.

2) If N changes stochastically around its mean $\overline{N}$ with sufficiently small deviations (compared with $\overline{N}$) and if these deviations are mutually independent, N in the previous formulas should be replaced by $\overline{N} - (V_N/\overline{N})$, where V_N is the variance of N.

Random Genetic Drift in a Tri-allelic Locus

In a randomly mating population of effective size N, let x_t, y_t and z_t $(=1-x_t-y_t)$ be the frequencies of three alleles denoted by A_1, A_2 and A_3 respectively in the t^{th} generation. As in the case of two alleles, we define a function $f(x,y;p_1,p_2;t)$ giving the probability that the frequencies of A_1 and A_2 are respectively x and y at the t^{th} generation when they start with $x = p_1$ and $y = p_2$ at the 0^{th} generation.

Let δx_t and δy_t be the amount of change in x_t and y_t due to the random sampling of gametes to form the next generation where frequencies of A_1 and A_2 will be x_{t+1} and y_{t+1}, then

$$x_{t+1} = x_t + \delta x_t, \qquad y_{t+1} = y_t + \delta y_t.$$

In order to find the recurrence formula which gives the law of change in the moments of distribution, we note that x_{t+1} and y_{t+1} are random variables from a trinomial distribution.

If we denote by $\mu'^{(t)}_{m,n}$ the m, n^{th} moment of distribution about zero at the t^{th} generation such that $\mu'^{(t)}_{m,n} = E(x_t^m y_t^n)$, then

$$\mu'^{(t+1)}_{m,n} = E\{(x_t + \delta x_t)^m (y_t + \delta y_t)^n\}.$$

Expanding the right side and noting that

$$E(\delta x_t) = E(\delta y_t) = 0, \qquad E(\delta x_t)^2 = x_t(1 - x_t)/2N,$$
$$E(\delta y_t)^2 = y_t(1 - y_t)/2N, \qquad E(\delta x_t \delta y_t) = -x_t y_t/2N,$$

we obtain the following recurrence formula, under the assumption that the population number N is sufficiently large that the terms of order $1/N^2$, $1/N^3$ etc., can be neglected without serious error:

$$\mu'^{(t+1)}_{m,n} = \left\{1 - \frac{m(m-1)}{4N} - \frac{mn}{2N} - \frac{n(n-1)}{4N}\right\} \mu'^{(t)}_{m,n} + \frac{m(m-1)}{4N} \mu'^{(t)}_{m-1,n} + \frac{n(n-1)}{4N} \mu'^{(t)}_{m,n-1} \quad (m, n \geq 1).$$

Under this assumption, the moments change very slowly per generation and we can replace the above equation by a system of differential equations:

$$\frac{d\mu'^{(t)}_{m,n}}{dt} = -\frac{(m+n)(m+n-1)}{4N} \mu'^{(t)}_{m,n} + \frac{m(m-1)}{4N} \mu'^{(t)}_{m-1,n} + \frac{n(n-1)}{4N} \mu'^{(t)}_{m,n-1}$$
$$(m, n = 1, 2, 3, \cdots).$$

If the initial frequencies of A_1, A_2 and A_3 in the population are p_1, p_2 and p_3 respectively $(p_1 + p_2 + p_3 = 1)$, $\mu'^{(0)}_{m,n} = p_1^m p_2^n$ and the first few moments are obtained directly.

$$
\left.
\begin{aligned}
\mu'^{(t)}_{1,1} &= p_1p_2e^{-(1/2N)t} \\
\mu'^{(t)}_{1,2} &= \tfrac{1}{2}p_1p_2e^{-(1/2N)t} + (p_1p_2^2 - \tfrac{1}{2}p_1p_2)e^{-(3/2N)t} \\
\mu'^{(t)}_{1,3} &= \tfrac{3}{10}p_1p_2e^{-(1/2N)t} + (p_1p_2^2 - \tfrac{1}{2}p_1p_2)e^{-(3/2N)t} \\
&\qquad + (p_1p_2^3 + \tfrac{1}{5}p_1p_2 - p_1p_2^2)e^{-(6/2N)t} \\
\mu'^{(t)}_{1,4} &= \tfrac{1}{5}p_1p_2e^{-(1/2N)t} + (6/7)(p_1p_2^2 - \tfrac{1}{2}p_1p_2)e^{-(3/2N)t} \\
&\qquad + \tfrac{3}{2}(p_1p_2^3 + \tfrac{1}{5}p_1p_2 - p_1p_2^2)e^{-(6/2N)t} \\
&\qquad + (p_1p_2^4 - \tfrac{3}{2}p_1p_2^3 + (9/14)p_1p_2^2 - (1/14)p_1p_2)e^{-(10/2N)t} \\
\mu'^{(t)}_{2,2} &= \tfrac{1}{5}p_1p_2e^{-(1/2N)t} + \tfrac{1}{3}(-p_1p_2p_3)e^{-(3/2N)t} \\
&\qquad + \left(p_1^2p_2^2 + \frac{p_1p_2p_3}{3} - \frac{p_1p_2}{5}\right)e^{-(6/2N)t} \\
\mu'^{(t)}_{2,3} &= \tfrac{1}{10}p_1p_2e^{-(1/2N)t} + (1/7)(2p_1p_2^2 + p_1^2p_2 - \tfrac{3}{2}p_1^2p_2)e^{-(3/2N)t} \\
&\qquad + \tfrac{1}{4}(\tfrac{3}{5}p_1p_2 + p_1p_2^3 - 2p_1p_2^2 - p_1^2p_2 + 3p_1^2p_2^2)e^{-(6/2N)t} \\
&\qquad + (p_1^2p_2^3 - \tfrac{3}{4}p_1^2p_2^2 - \tfrac{1}{4}p_1p_2^3 + (3/14)p_1p_2^2 \\
&\qquad + (3/28)p_1^2p_2 - (1/28)p_1p_2)e^{-(10/2N)t}.
\end{aligned}
\right\} \quad (12)
$$

By virture of symmetry, $\mu'^{(t)}_{2,1}$, $\mu'^{(t)}_{3,1}$, etc. can be obtained simply by interchanging p_1 and p_2 with each other. These formulas may be used for treating observational results on natural populations. The same type of formulas have been derived by Robertson (1952, p. 205) for the case of a pair of alleles.

From the above derivation it will be seen that the general moment formula must have the form:

$$\mu'^{(t)}_{m,n} = C^{(1)}_{m,n}e^{-(1/2N)t} + C^{(2)}_{m,n}e^{-(3/2N)t} + \cdots + C^{(m+n-1)}_{m,n}e^{-[(m+n)(m+n-1)/4N]t}. \quad (13)$$

Substituting this back in (11) and solving the resulting finite difference equations with respect to these coefficients, we obtain the following expressions:

$$C^{(1)}_{m,n} = \frac{6\cdot m!n!}{(m+n+1)!}C^{(1)}_{1,1}$$

$$C^{(2)}_{m,n} = \frac{60\cdot m!n!}{(m+n+2)!}\{(m-1)C^{(2)}_{2,1} + (n-1)C^{(2)}_{1,2}\}$$

$$C^{(3)}_{m,n} = \frac{420\cdot m!n!}{(m+n+3)!}\{(m-1)(m-2)C^{(3)}_{3,1} + 3(m-1)(n-1)C^{(3)}_{2,2} + (n-1)(n-2)C^{(3)}_{1,3}\}$$

$$C^{(4)}_{m,n} = \frac{9!}{3!4!}\frac{m!n!}{(m+n+4)!}\{(m-1)(m-2)(m-3)C^{(4)}_{4,1} + 6(m-1)(m-2)(n-1)C^{(4)}_{3,2} + 6(m-1)(n-1)(n-2)C^{(4)}_{2,3} + (n-1)(n-2)(n-3)C^{(4)}_{1,4}\} \text{ etc.},$$

where the coefficients in the right side are the coefficients of the moment formulas given in (12). For example, $C^{(1)}_{1,1}$ is the first coefficient in $\mu'^{(t)}_{1,1}$ which is p_1p_2. As t gets large the exponential terms in (13) soon become very small so that only the first few terms are important.

First let us find the probability that the gene A_1, A_2 or A_3 becomes fixed in the population by the t^{th} generation. For this purpose we need only put $p = p_1$, p_2 or p_3 in the formula $f(1;p;t)$ in (1) which gives the same kind of probability for the case of two alleles. The function $f(1;p;t)$ tells us more about the process of the change

in the triallelic case. Let $P_t(A_1)$ be the probability of A_1 being fixed in the population by the t^{th} generation, $P_t(A_1A_2)$ the probability that A_1 and A_2 coexist in the population but A_3 is lost from it, and $P_t(A_1A_2A_3)$ be the probability that 3 genes A_1A_2 and A_3 still coexist in the population in the t^{th} generation. Then

$$P_t(A_1) = f(1;p_1;t), \quad P_t(A_2) = f(1;p_2;t), \quad P_t(A_3) = f(1;p_3;t)$$

$$P_t(A_1A_2) = f(1;p_1 + p_2;t) - f(1;p_1;t) - f(1;p_2;t)$$

$$P_t(A_1A_2A_3) = 1 - P_t(A_1) - P_t(A_2) - P_t(A_3) - P_t(A_1A_2) - P_t(A_1A_3) - P_t(A_2A_3).$$

From these relations we obtain

$$P_t(A_1A_2) = 6p_1p_2e^{-(1/2N)t} - 30p_1p_2p_3e^{-(3/2N)t} + 14p_1p_2\{1 - 5(1 - p_1)(1 - p_2) + 10p_3^2\}e^{-(6/2N)t} + \cdots \quad (14)$$

$$P_t(A_1A_2A_3) = 60p_1p_2p_3e^{-(3/2N)t} + 90p_1p_2p_3\{7(p_1^2 + p_2^2 + p_3^2) - 3\}e^{-(10/2N)t} + \cdots. \quad (15)$$

These formulas tell us that at the final state of steady decay, the frequency of the classes in which only two alleles coexist decreases at the rate of $1/2N$, while that of classes in which the 3 alleles coexist decreases at the rate of $3/2N$ per generation.

This fact suggests that the moment formula (13) contains contributions from these heterogeneous classes. So we shall start our consideration from the distribution of gene frequencies among the classes where the 3 alleles coexist. As in the case of a pair of alleles, the gene frequencies x and y are discrete random variables which take on values between 0 and 1 with steps of size $1/2N$, but we introduce a simplifying assumption that N is sufficiently large that we can treat x and y as continuous random variables with good approximation. Of course, we must assume discontinuity of the distribution at the terminal part because of the irreversible process by which alleles become fixed or lost by chance.

Let $\phi(x,y;p_1,p_2;t)$ be the probability density that the frequency of A_1 lies between x and $x + dx$ and at the same time A_2 lies between y and $y + dy$ in the t^{th} generation $(0<x<x+y<1)$, given that one starts from $x = p_1$ and $y = p_2$ at $t = 0$. The probability $\phi(x,y;p_1,p_2;t)dxdy$ is a substitute for $f(x,y;p_1,p_2;t)$ with dx and dy each being substituted for $1/2N$. In order to obtain this, we need the moment for this distribution which does not contain the classes where any one of the alleles has been lost. Actually we need only to remove from (13) the contribution from the classes where the gene A_3 has been lost, since (13) vanishes automatically for other cases of $x = 0$ (A_1 lost) and $y = 0$ (A_2 lost). For this purpose consider the quantity:

$$\nu^{(t)}_{m,n,k} = \sum_{x=0}^{1}\sum_{y=0}^{1} x^m y^n (x + y)^k f(x,y;p_1,p_2;t), \quad (16)$$

by letting x and y discrete variables in the right side.

(16) can be calculated by (13) through the relation:

$$\nu^{(t)}_{m,n,k} = E\{x^m y^n (x + y)\}^k = \sum_{i=0}^{1} \frac{k!}{i!(k-i)!}\, \mu'^{(t)}_{m+1,n+k-1}.$$

What we are going to use is the property of (16) that by letting $k \to \infty$, terms in the right hand side of (16) vanish except for the case when $x + y = 1$ i.e., $z = 0$. Thus

$$\lim_{k\to\infty} \nu^{(t)}_{m,n,k} = \sum_{x+y=1} x^m y^n f(x,y;p_1,p_2;t) \quad (17)$$

is the part to be removed from (13). The explicit expression of (17) is as follows;

$$\lim_{k\to\infty} \nu^{(t)}_{m,n,k} = D^{(1)}_{m,n}e^{-(1/2N)t} + D^{(2)}_{m,n}e^{-(3/2N)t} + D^{(3)}_{m,n}e^{-(6/2N)t} + D^{(4)}_{m,n}e^{-(10/2N)t} + \cdots, \quad (18)$$

where

$$D^{(1)}_{m,n} = \frac{6\cdot m!n!}{(m+n+1)!}C^{(1)}_{1,1}$$

$$D^{(2)}_{m,n} = \frac{60\cdot m!n!}{(m+n+2)!}\{(m+1)C^{(2)}_{2,1} + (n+1)C^{(2)}_{1,2}\}$$

$$D^{(3)}_{m,n} = \frac{420\cdot m!n!}{(m+n+3)!}\{(m+1)(m+2)C^{(3)}_{3,1} + 3(m+1)(n+1)C^{(3)}_{2,2} + (n+1)(n+2)C^{(3)}_{1,3}\}$$

$$D^{(4)}_{m,n} = \frac{9!}{3!4!}\frac{m!n!}{(m+n+4)!}\{(m+1)(m+2)(m+3)C^{(4)}_{4,1} + 6(m+1)(m+2)(n+1)C^{(4)}_{3,2} + 6(m+1)(n+1)(n+2)C^{(4)}_{2,3} + (n+1)(n+2)(n+3)C^{(4)}_{1,4}\} \text{ etc.}$$

Thus the required moment is obtained by subtracting (18) from (13):

$$\int_0^1\int_0^{1-y} x^m y^n \phi(x,y;p_1,p_2;t)dxdy = \mu'^{(t)}_{m,n} - \lim_{k\to\infty}\nu^{(t)}_{m,n,k}$$
$$= E^{(1)}_{m,n}e^{-(1/2N)t} + E^{(2)}_{m,n}e^{-(3/2N)t} + E^{(3)}_{m,n}e^{-(6/2N)t} + E^{(4)}_{m,n}e^{-(10/2N)t} + \cdots, \quad (19)$$

where

$$E^{(1)}_{m,n} = 0$$

$$E^{(2)}_{m,n} = -\frac{120\cdot m!n!}{(m+n+2)!}\{C^{(2)}_{2,1} + C^{(2)}_{1,2}\}$$

$$E^{(3)}_{m,n} = -\frac{7!}{2!}\frac{m!n!}{(m+n+3)!}\{m(C^{(3)}_{3,1} + C^{(3)}_{2,2}) + n(C^{(3)}_{2,2} + C^{(3)}_{1,3})\}$$

$$E^{(4)}_{m,n} = -\frac{9!}{3!2!}\frac{m!n!}{(m+n+4)!}\{(m^2+1)C^{(4)}_{4,1} + (3mn+m^2+2)C^{(4)}_{3,2} + (3mn+n^2+2)C^{(4)}_{2,3} + (n^2+1)C^{(4)}_{1,4}\} \text{ etc.}$$

Applying the relations:

$$\frac{m!n!}{(m+n+2)!} = \int_0^1\int_0^{1-y} x^m y^n dxdy$$

$$\frac{m!n!}{(m+n+3)!} = \int_0^1\int_0^{1-y} x^m y^n z dxdy$$

$$\frac{m!n!}{(m+n+4)!} = \frac{1}{2}\int_0^1\int_0^{1-y} x^m y^n z^2 dxdy$$

etc. to (19), we arrive at the desired formula which gives the simultaneous frequency distribution of A_1 and A_2:

$$\phi(x,y;p_1,p_2;t) = 5!p_1p_2p_3e^{-(3/2N)t} + \frac{7!}{2!}p_1p_2p_3\{(p_1 - \tfrac{1}{3})x + (p_2 - \tfrac{1}{3})y + (p_3 - \tfrac{1}{3})z\}e^{-(6/2N)t} + \frac{9!}{3!2!}p_1p_2p_3\left\{(p_1^2 - \tfrac{3}{4}p_1 + 3/28)x^2 + (p_2^2 - \tfrac{3}{4}p_2 + 3/28)y^2 + (p_3^2 - \tfrac{3}{4}p_3 + 3/28)z^2 + 3\left(p_1p_2 - \frac{p_1 + p_2}{4} + \frac{1}{14}\right)xy + 3\left(p_1p_3 - \frac{p_1 + p_3}{4} + \frac{1}{14}\right)xz + 3\left(p_2p_3 - \frac{p_2 + p_3}{4} + \frac{1}{14}\right)yz\right\}e^{-(10/2N)t} + \cdots, \quad (20)$$

where $z = 1 - x - y$ and $p_3 = 1 - p_1 - p_2$. The final rate of decay is $3/2N$ per generation as was inferred from (15). By direct integration we can verify that

$$P_t(A_1A_2A_3) = \int_0^1 \int_0^{1-y} \phi(x,y,p_1,p_2;t)dxdy.$$

For the purpose of illustrating the process of change in the frequency distribution of various classes, triangular coordinates will be convenient. If we start from a population that contains 3 alleles A_1, A_2 and A_3 with frequencies $p_1 = 0.1$, $p_2 = 0.3$ and $p_3 = 0.6$ respectively, the initial population is expressed as a point Q in figure 2. After N generations, the gene frequency distribution given by $\phi(x,y;0.1,0.3;N)$ form a surface on the triangular coordinate $A_1A_2A_3$ as illustrated by the cross-hatched surface in figure 3. In this case after 2N generations the surface becomes very nearly flat as is seen in the same figure (dotted surface). Volumes under these surfaces give probabilities $P_t(A_1A_2A_3)$ that 3 alleles coexist in the population at $t = N$ and $t = 2N$ respectively.

With the continuous model, it can be shown that $\phi(x,y;p_1,p_2;t)$ must satisfy the following partial differential equation:

$$\frac{\partial\phi}{\partial t} = \frac{1}{4N}\frac{\partial^2}{\partial x^2}\{x(1 - x)\phi\} - \frac{1}{2N}\frac{\partial^2}{\partial x\partial y}\{xy\phi\} + \frac{1}{4N}\frac{\partial^2}{\partial y^2}\{y(1 - y)\phi\}. \quad (21)$$

By the direct substitution of (20) into (21), it has been verified that (20) satisfies this equation.[2]

Next, let us designate by $\phi_{12}(x;t)$ the probability density in the t^{th} generation, that the frequency of gene A_1 lies between x and $x + dx$ $(0 < x < 1)$, with A_1 and A_2 coexisting but A_3 has been lost from the population.

This probability density can be obtained from (18) since

$$\int_0^1 x^m(1 - x)^n\phi_{12}(x;t)dx = \lim_{k\to\infty} \nu^{(t)}_{m,n,k}.$$

By using the relation:

$$\frac{m!n!}{(m + n + 1)!} = \int_0^1 x^m(1 - x)^n dx,$$

[2] Since this paper was written, the complete solution of (21) has been obtained. The result confirmed formula (20). The detailed solution is to be published in Biometrics: Kimura, M. "Random genetic drift in a tri-allelic locus: Exact solution with a continuous model."

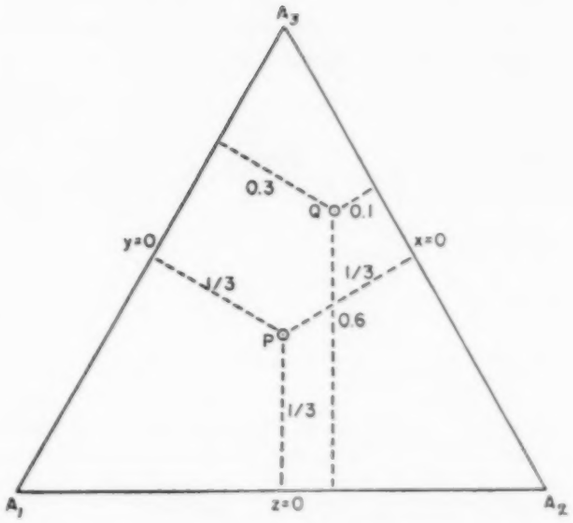

FIG. 2. Expression of the gene frequencies of the populations on the triangular coordinate.

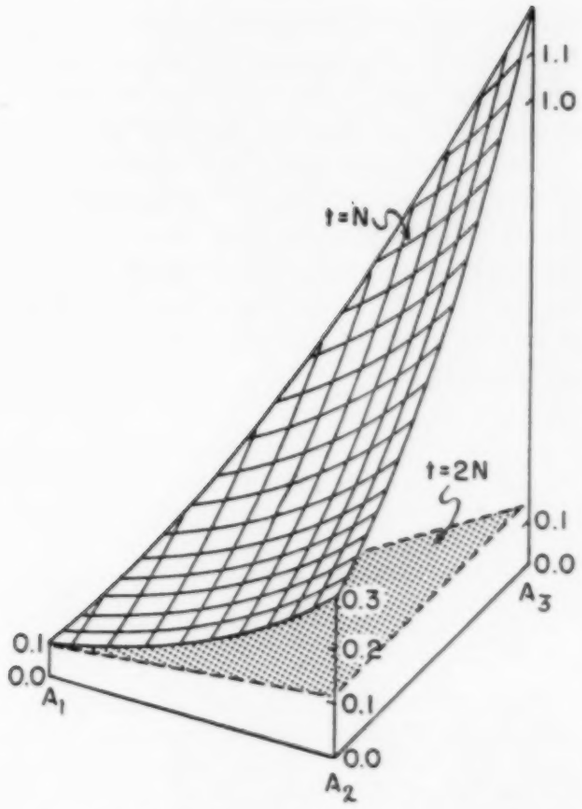

FIG. 3. Surfaces on the triangular coordinate, showing the frequency distributions of the classes where all the three alleles A_1, A_2 and A_3 coexist. In this case the initial gene frequencies are $p_1 = 0.1$, $p_2 = 0.3$ and $p_3 = 0.6$, i.e. the population starts from the point Q in the fig. 2. The cross-hatched and the dotted surfaces show respectively the distributions after N and 2N generations, where N is the effective population size.

we obtain

$$\phi_{12}(x;t) = 3!C_{1,1}^{(1)}e^{-(1/2N)t} + \frac{5!}{2!}\{C_{2,1}^{(2)}x + C_{1,2}^{(2)}(1-x)\}e^{-(3/2N)t}$$
$$+ \frac{7!}{3!2!}\{C_{3,1}^{(3)}x^2 + 3C_{2,2}^{(3)}x(1-x) + C_{1,3}^{(3)}(1-x)^2\}e^{-(6/2N)t}$$
$$+ \frac{9!}{4!3!}\{C_{4,1}^{(4)}x^3 + 6C_{3,2}^{(4)}x^2(1-x)$$
$$+ 6C_{2,3}^{(4)}x(1-x)^2 + C_{1,4}^{(4)}(1-x)^3\}e^{-(10/2N)t} + \cdots. \quad (22)$$

In this case the final rate of decay is $1/2N$ per generation as was inferred from (14). By direct integration of (22), we can verify that

$$P_t(A_1A_2) = \int_0^1 \phi_{12}(x;t)dx.$$

The probability density, $\phi_{23}(y;t)$, $(0 < y < 1)$, giving the frequency distribution of the gene A_2 when A_1 is lost can be obtained from (22) by replacing p_1, p_2 and p_3 with p_2, p_3 and p_1 respectively. Similarly the density, $\phi_{31}(z;t)$, $(0 < z < 1)$, giving

the frequency distribution of the gene A_3 when A_2 is lost can be obtained from (22) by replacing p_1, p_2 and p_3 with p_3, p_1 and p_2 respectively. These formulas together with (20) and (1) in which p is replaced by p_1, p_2 and p_3 successively can completely describe the process of random genetic drift in a tri-allelic locus.

Some examples are illustrated in fig. 4 and fig. 5. In fig. 4 the population starts from the gene frequency corresponding to P in fig. 2, while in fig. 5, the population starts from the point Q. It is interesting to observe that though $\phi(x,y,p_1,p_2;t)$ becomes practically flat in both cases after 2N generations, distributions given by $\phi_{12}(x,t)$, $\phi_{23}(y,t)$ etc., are widely different in both cases depending on the starting point in gene frequency. All distributions, however, become finally flat after a sufficient time.

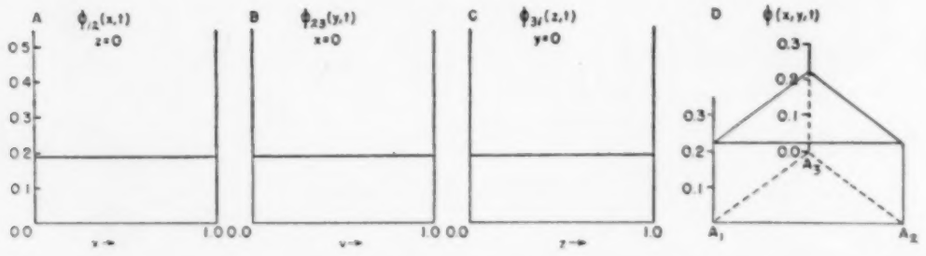

FIG. 4 A–D. Various frequency distributions for different classes after 2N generations assuming that the initial composition of the population is $p_1 = p_2 = p_3 = \frac{1}{3}$, i.e. the population starts from the point P in the fig. 2. For example, A shows the frequency distribution of the classes where A_1 and A_2 coexist but A_3 is lost from the population. Also, *D* shows the frequency distribution of the classes where all three alleles coexist. x, y and z denote respectively the frequencies of A_1, A_2 and A_3. The probabilities of the various states at t = 2N are as follows:

$$P(A_1) = P(A_2) = P(A_3) \doteqdot 10.7\%,$$
$$P(A_1A_2) = P(A_2A_3) = P(A_3A_1) \doteqdot 19.0\%,$$
$$P(A_1A_2A_3) = 11.0\%.$$

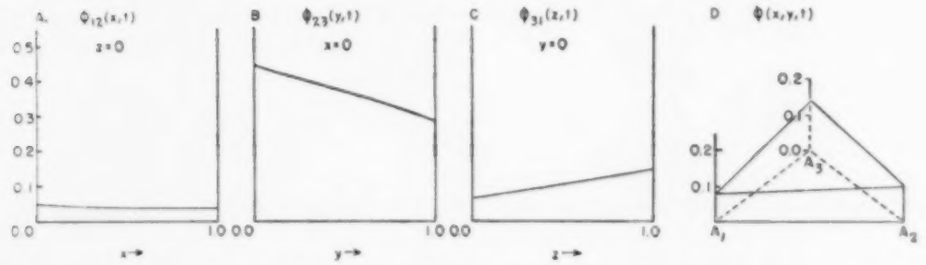

FIG. 5. A–D. Same as the fig. 4 except for the population starts from $p_1 = 0.1$, $p_2 = 0.3$ and $p_3 = 0.6$, i.e. from the point Q in the fig. 2. The probabilities of the various states at t = 2N are:

$$P(A_1) = 1.8\%, \quad P(A_2) = 8.9\%, \quad P(A_3) = 32.4\%,$$
$$P(A_1A_2) = 4.1\%, \quad P(A_2A_3) = 36.8\%, \quad P(A_1A_3) = 10.6\%,$$
$$P(A_1A_2A_3) = 5.4\%, \text{ approximately.}$$

To summarize the process of steady decay, fig. 6 has been constructed.

The 3 columns standing on the apexes A_1, A_2 and A_3 represent the frequencies of 3 fixed classes and increase in height as fixation of alleles proceeds. The plane $B_1B_2B_3$ represents the distribution of the classes where 3 alleles coexist and this plane declines at the rate of 3/2N per generation. The 3 surrounding walls each of which represents the distribution of two alleles when the third is lost, decreases in height at the rate of 1/2N per generation. The upper edge of each of them is flat but their heights are not necessarily the same.

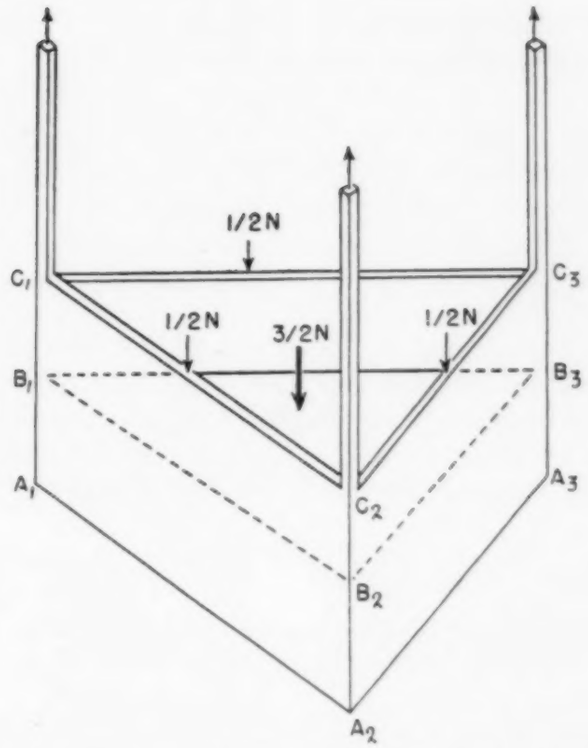

FIG. 6. Explanatory figure to summarize the results obtained for the triallelic case. The relative proportion of areas and the volume in the figure are not exact.

Any population which is located in an interior point of the co-ordinate triangle $A_1A_2A_3$ (see fig. 2) may wander randomly from generation to generation and if it happens to reach one of the edges, say A_1A_2, then it can wander only along it. Finally the point may reach one of its terminal points, say A_1, and stop moving, which means that gene A_1 is fixed.

The decrease of heterozygosis is a different matter. As in the case of a pair of alleles, the heterozygosis decreases exactly at the rate of $1/2N$ per generation from the beginning, as is easily demonstrated by a procedure similar to that employed in the case of a pair of alleles: Let p_1, p_2 and p_3 be the frequencies of A_1, A_2 and A_3 in the population where the frequency of heterozygotes is $\sum_{j>i} 2p_ip_j$ $(i, j = 1, 2, 3)$. After one generation of random mating, the frequency of heterozygosis in the next generation is expected to be

$$E\{2\sum_{i>j}(p_i + \delta p_i)(p_j + \delta p_j)\} = 2\sum_{i>j} E(p_ip_j) + 2\sum_{i>j} E(\delta p_i \delta p_j)$$

$$= 2\sum E(p_ip_i) + 2\sum E\left(\frac{-p_ip_j}{2N}\right) = \{2\sum_{i>j} p_ip_j\}\left(1 - \frac{1}{2N}\right).$$

Therefore

$$H_t = H_0 e^{-(1/2N)t}.$$

GENERAL CASE OF MULTIPLE ALLELES

From the foregoing treatment one might infer that in the general case of m alleles, also the "flat" distribution of heterallelic classes will be realized after sufficiently many generations. At this state of steady decay, the probability density $\phi(x_1, \cdots, x_{m-1}; p_1, \cdots, p_{m-1}; t)$ that all the original m alleles coexist in the population will decrease at the rate of $m(m-1)/4N$ per generation, where x_i is the frequency

of the i^{th} allele whose initial frequency is p_i ($\sum p_i = 1$). This can be verified from the Fokker-Planck equation for the case of the m alleles:

$$\frac{\partial \phi}{\partial t} = \frac{1}{4N} \sum_{i=1}^{m-1} \frac{\partial^2}{\partial x_i^2} \{x_i(1 - x_i)\phi\} - \frac{2}{4N} \sum_{i>j} \frac{\partial^2}{\partial x_i \partial x_j} \{x_i x_j \phi\}$$
$$(i, j = 1, 2, \cdots, m - 1), \quad (23)$$

which is satisfied by

$$\phi = C \cdot e^{-[m(m-1)/4N]t}. \quad (24)$$

The constant C is determined as follows: Let $H_t^{(m)}$ be the probability that m randomly chosen genes are all different. For large N, this probability is proved to decrease at the rate of approximately $\binom{m}{2}/2N$ per generation, which is independent of t. To prove this we let p_i ($i = 1, \cdots, m$) be the frequency of the gene A_i in the population. Then the same probability in the next generation is expected to be proportional to

$$E\{(p_1 + \delta p_1)(p_2 + \delta p_2) \cdots (p_m + \delta p_m)\}$$
$$= E\left\{ \prod_{i=1}^{m} p_i + \sum_{j=1}^{m} \left(\prod_{i \neq j} p_i\right)\delta p_j + \sum_{(j \neq k)} \left(\prod_{i \neq j,k} p_i\right)\delta p_j \delta p_k + \text{etc.} \right\}$$
$$= \prod_{i=1}^{m} p_i + \sum_{(j \neq k)} \left(\prod_{i \neq j,k} p_i\right) E(\delta p_j \delta p_k) + O(1/N^2) \doteqdot \{1 - \binom{m}{2}/2N\} \prod_{i=1}^{m} p_i.$$

Hence

$$H_t^{(m)} = \left\{1 - \frac{m(m-1)}{4N}\right\} H_{t-1}^{(m)},$$

if we neglect the terms of $O(1/N^2)$. Thus

$$H_t^{(m)} = m! \left(\prod_{i=1}^{m} p_i\right) e^{-[m(m-1)/4N]t}, \quad (25)$$

since $H_0^{(m)} = m! \prod p_i$.

On the other hand, this probability is also obtained by

$$m! \int\int \cdots \int_{x_1 + x_2 + \cdots + x_m = 1} \left(\prod_{i=1}^{m} x_i\right) \phi(x_1, \cdots, x_{m-1}; p_1, \cdots, p_{m-1}; t) \prod_{i=1}^{m-1} dx_i.$$

Using (24), we obtain the asymptotic relation:

$$H_t^{(m)} \sim C \cdot \frac{m!}{(2m-1)!} e^{-[m(m-1)/4N]t}. \quad (26)$$

By comparing this with (25), we find $C = (2m - 1)! \prod_{i=1}^{m} p_i$ so that

$$\phi(x_1, \cdots, x_{m-1}; p_1, \cdots, p_{m-1}; t) \sim (2m - 1)! \left(\prod_{i=1}^{m} p_i\right) e^{-[m(m-1)/4N]t}. \quad (27)$$

The probability that the m alleles coexist in the population at the t^{th} generation is obtained by

$$\Omega_t^{(m)} = \int\int \cdots \int_{x_1 + x_2 + \cdots + x_m = 1} \phi(x_1, \cdots, x_{m-1}; p_1, \cdots, p_{m-1}; t) \prod_{i=1}^{m-1} dx_i$$

which is asymptotically

$$\Omega_t^{(m)} \sim \frac{(2m-1)!}{(m-1)!}\left(\prod_{i=1}^{m} p_i\right)e^{-[m(m-1)/4N]t}. \tag{28}$$

In the case of the distributions of heteroallelic classes where some alleles have been lost, the Fokker-Planck equation does not hold exactly since contributions may also come from other classes containing additional unfixed alleles. The equation is applicable only asymptotically and we have the following general result:

If we start from the population which contains m alleles say $A_1, A_2, \cdots, A_m$ with frequencies $p_1, p_2 \cdots p_m$ $(\sum_1^m p_i = 1)$, the probability density that it contains k of them say $A_1, A_2, \cdots$ and A_k with frequencies $x_1, x_2, \cdots$ and x_k $(\sum_1^k x_i = 1)$ in the t^{th} generation is given asymptotically by

$$\phi_{1,2,\cdots k}(x_1, x_2, \cdots, x_{k-1}, t) \sim (2k-1)!\left(\prod_{j=1}^{k} p_j\right)e^{-[k(k-1)/4N]t}, \tag{29}$$

where $k \leq m$. The validity of this formula depends on the assumption that the population size N is sufficiently large as compared with m, the number of the alleles in question.

On the other hand the amount of heterozygosis always decreases at the rate of $1/2N$:

$$H_t = H_0 e^{-(1/2N)t}, \tag{30}$$

which can easily be shown by exactly the same method employed in the case of 3 alleles.

The effectiveness of random genetic drift as an evolutionary factor in natural populations is still a problem of much dispute (Fisher and Ford, 1947; Wright, 1948; Fisher and Ford, 1950; Wright, 1951). Since the purpose of this paper is to present theoretically the general picture of the process of random genetic drift in an idealized situation, the present writer has no intention to join this discussion. It must be noted, however, that as the number of the coexisting alleles increases, the rate at which the probability of coexistence is reduced increases rapidly as (29) indicates. In this sense, the effect of the random genetic drift might have an important bearing on the genetic structure of natural populations by keeping down the number of coexisting alleles in the population.

The causes of random fluctuation of gene frequency in natural populations are not restricted to the random sampling of gametes in reproduction to which exclusive attention has been paid in this paper. Another important factor is the random fluctuation of the selection intensity. The process of the change in gene frequency due to the latter factor in an idealized condition was investigated earlier (Kimura, 1954). The steady state gene frequency distribution involving this factor was first worked out by Wright (1948).

In natural populations, these two factors must be working simultaneously, and, in conjunction with mutation, selection and migration, constitute a main factor in evolution.

Summary

After the short review of the theoretical as well as experimental results so far obtained on the problem of the random genetic drift due to small population number,

the complete solution of the process of the random genetic drift for the case of a pair of alleles is illustrated.

From this it is pointed out that the well known steady decay solution; $\phi = Ce^{-t/2N}$, though useful when the initial gene frequency is near 50%, decreases in usefulness as the initial gene frequency deviates from this value (fig. 1a and 1b).

The main purpose of this paper, however, is to present the general treatment of the problem of random drift for a multiallelic locus. First the asymptotic solutions are elaborated for the triallelic system. They show that as the number of generations becomes large, the rate of decay of the frequencies of the classes where only two alleles coexist approaches 1/2N per generation, while that of the classes where the 3 alleles coexist approaches 3/2N per generation.

The most general result obtained is as follows: If we start from a population which contains m alleles say $A_1, A_2, \cdots$ and A_m with frequencies $p_1, p_2, \cdots$ and p_m respectively ($\sum p_i = 1$), the probability density that it contains k of them, say $A_1, A_2, \cdots$ and A_k with respective frequencies $x_1, x_2, \cdots$ and x_k ($\sum_1^k x_i = 1$) in the t^{th} generation is given asymptotically by

$$\phi_{1,2,\cdots,k}(x_1,x_2,\cdots,x_{k-1};t) \sim (2k-1)!\left(\prod_{j=1}^{k} p_j\right) \exp\{-k(k-1)t/4N\},$$

where $k < m$. This indicates that as the number of coexisting alleles increases, the rate by which that state is eliminated increases with rapid progression. In this sense, random drift might be effective in keeping down the number of coexisting alleles in the population.

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LOCAL INTROGRESSION BETWEEN *VIOLA CUCULLATA* AIT. AND *V. SEPTENTRIONALIS* GREENE

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INTRODUCTION

Two commonly found, stemless [1] blue violets in New England are *Viola cucullata* Ait. and *V. septentrionalis* Greene. Both grow in essentially northern forest habitats, *V. cucullata* in wet soil beside cold springs, bogs, or woodland streams, and *V. septentrionalis* in drier locations, usually beneath conifers. Where found, both species may be abundant, but tend to grow as scattered plants rather than in dense clonal clusters, as is often true of such related species as *V. sororia* and *V. papilionacea*. Though their habitats are usually not contiguous, populations of the two species are frequently found side by side, due to disturbance. This is particularly true along creeks running through partly logged or grazed hemlock woods. In these locations *V. cucullata* occurs on the wet sand or mud at the water's edge and *V. septentrionalis* on the drier creek bank and in the hemlock forest.

V. cucullata has been collected from Newfoundland to the Thunder Bay District of Ontario, and south along the uplands to Minnesota and Georgia. *V. septentrionalis* has a somewhat similar distribution, occurring from Newfoundland to southern British Columbia and south to Tennessee in the mountains (Fernald, 1950). The ranges of the two species overlap greatly with each other, and also coincide, at least in part, with the ranges of distribution of a number of other species of the Subsection *Boreali-Americanae* of *Viola*, the so-called stemless blue violets. All these violets have chromosome numbers of 2n = 54 (Gershoy, 1934) and apparently are very closely related—so much so that many different hybridizations between them have been reported in nature. For example, Fernald (1950) lists *V. cucullata* as crossing with *V. papilionacea, V. nephrophylla, V. affinis, V. sororia, V. septentrionalis, V. fimbriatula, V. sagittata, V. triloba, V. palmata, V. Brittoniana,* and *V. viarum*. *V. septentrionalis* is said to cross with *V. cucullata, V. affinis, V. sororia, V. fimbriatula,* and *V. palmata*. Brainerd (1904, 1906, 1924) reported upon a number of these naturally occurring crosses. His studies of assumed F_1 hybrids and of later generations obtained from these putative F_1 plants indicate that backcrossing and introgression might often follow the initial crosses. As might be expected, perhaps the chief deterrent to studies of variation in these violets is the great difficulty of defining the "pure" or original phenotype of each species. Often intermediate or nondescript specimens will outnumber easily determined ones in field populations . Gershoy (1934) lists a large number of experimental F_1 hybrids in the *Boreali-Americanae*. His studies do not include analysis of segregation in later generations, although he indicates multiple factor inheritances in these wild species.

During the summer of 1953 the author was the resident fellow on the Edmund Niles Huyck Preserve. This 500-acre Preserve is located near Rensselaerville in Albany County, New York, and was established in 1931 in memory of Mr. Huyck, and ". . . To preserve the natural beauty of the Rensselaerville Falls, Lake

[1] The "stemless" or "acaulescent" violets actually possess well-developed stems, but they are almost completely underground. Division of the genus *Viola* into "stemless" and "stemmed" groups of species apparently follows evolutionary trends.

Myosotis, Lincoln Pond and the lands around them in the Town of Rensselaerville, Albany County, New York. . . ." The author is deeply indebted to the Directors of the Huyck Preserve for supporting the research reported upon here.

The Huyck Preserve is located on the Helderberg Plateau at an elevation varying between 1550 and 1700 feet. Within its confines eleven species of violets were found, three of which were stemless blue violets: *V. cucullata, V. septentrionalis,* and *V. fimbriatula.* In the immediate vicinity of the Preserve (within a ten-mile radius) only one other species of stemless blue violet, *V. sororia,* was found by the author. Both *V. sororia* and *V. fimbriatula* were infrequent and very rarely found near the other two violets. As a result of this distribution an excellent opportunity was presented to observe the effects of two species of stemless blue violets upon each other in relative genetic isolation. The principal purpose of this study is to determine the morphological characteristics of these two species prior to the onset of introgression between them.

Methods

Twenty-six population samples of *V. cucullata* and 24 of *V. septentrionalis* were taken during June, July, and August, 1953, on or near the E. N. Huyck Preserve. Sixteen representative collections of each species are discussed in this paper. Mature leaves only were studied, since in previous work (Russell, 1952; Russell and Kunimura, 1955) it had been found that leaf characters might be used as indices of total plant variation.

A single large, mature leaf was taken from each of 40 plants (or fewer, if the population was small) and either measured while in the field, or returned to the laboratory and measured the same day, while still fresh. Since there was a possibility of extensive root clones in both species, individual plants closer than about six feet were not sampled. In some cases the leaves were pressed and kept; in most instances they were discarded after measurements had been made.

Leaf measurements made were the following:

Lamina length along midrib.
Lamina breadth at broadest part of blade.
Length to lobe—the distance from the apex of the leaf to the tip of one of the basal lobes.
Number of teeth (crenations) on one half of the blade.
Lamina pubescence (presence or absence) on upper and lower surfaces and margins.
Petiole pubescence (presence or absence).

From these data several ratios were calculated. The computed ratio between length and breadth of the lamina is here considered to be a rough but fairly accurate indication of overall lamina shape in the violets. The ratio between length of the lamina and the distance from the leaf tip to the lobe measures a different aspect of leaf shape; namely, the development of basal lobes. Finally, since it was found that the number of teeth on one half of the lamina varied in direct relation to the size of the leaf, the ratio of lamina length/number of teeth was used and found much more significant than the number of teeth alone.

The Hybridization Index

One of the simpler and more widely used techniques for indicating introgression—the hybridization index (Anderson, 1949)—has been used in the analysis of data here. After the first few collections were made, the writer realized that these two species were hybridizing widely in this area. In nearly every population of either species, individual plants were found that resembled, in several characters, the other species. This was most frequently found to be the case along streams where the two species were contiguous. Frequently specimens would be taken which were almost exactly inter-

TABLE 1. *Characters used and values assigned in the computation of hybrid indices*

Character	Range and value	
	V. septentrionalis (=0)	*V. cucullata* (=2)
Ratio, length/breadth of leaf blades	0.91–1.35	0.50–0.90
Ratio, length of leaf blade/length of lobe	0.71–0.95	0.56–0.70
Ratio, length of leaf blade/no. teeth on one margin	2.01–3.50	1.01–2.00
Leaf blade pubescence	both surfaces pubescent	upper surface glabrous
Petiole pubescence		

mediate between the two supposed parent species. This was true not only in the measured leaf characters but in flower, fruit, and other vegetative characters also.

The first problem in the preparation of a hybridization index is the determination of the characteristics of the two parents. Often this is not difficult; all one must do is to find populations free or nearly free from introgression from related species. In the present instance populations of this sort of either species were not found. In the preparation of the hybridization index the ranges of variation assigned to each species were determined from a preliminary visual inspection of the data and from published taxonomic descriptions. Five leaf characters were chosen that were thought to have little overlap. Two of these concern leaf shape, two leaf pubescence, and one the lamina margin. In table 1 they are listed, together with the ranges and values for each parent. The values have been so assigned that a typical plant of *V. cucullata* will have a score of 10, a typical plant of *V. septentrionalis* a score of 0. Intermediate scores should indicate hybrids or backcrosses. Intermediate values were not assigned for individual characters, the method being considered not definitive enough in this case for separation of more than two categories.

Finally, each individual plant in each collection was scored by use of the hybridization index. The distributions of the scores in each collection and the collection means are given in tables 2 and 3 for the two species.

ANALYSIS OF NATURAL POPULATIONS

The distributions of scores in the collections show various degrees of introgression in both directions. Only a few collections for either species have a real preponderance of scores in the end columns. Collections 36, 47, 48, and 74 for *V. septentrionalis* may be said to be relatively little affected by genes from *V. cucullata*. On the other hand, collections 46, 52, 96, 114, and 122 are considerably affected, the median score being 4 instead of the expected 0 for this species.

For *V. cucullata* we find that introgression is slightly less evident. Collections 45, 70, 73, 104, and 112 are relatively unaffected. In collection 55, the median has been moved two places, to the value 6. Collection 90 is a special case. It was taken on a hillside between populations of *V. cucullata* (collection 91) and *V. fimbriatula* and very obviously represented a hybrid swarm between them.

Because of the nature of the hybridization index, a mean score of 5 would indicate a population exactly intermediate between pure populations of the parent species and one where F_1 plants would in all probability predominate. The closest approach to this mean score is found in population samples 96 (4.86) and 114 (4.24) of *V. septentrionalis*. Collection 90 of *V. cucullata* had a mean score of 4.29 but represented an unusual case, as mentioned above. The fact that more intermediate collections were not found may be explained in two ways. In spite of the lack of apparent genetic barriers to hybridization between these two species, crossing may be somewhat restricted; to attain a mean of 5 there must be either perfectly free interbreeding, or a balancing mixture of plants tending toward both parents in morphological characters. In the present case, the method of sampling may have been responsible for the lack of

exactly intermediate populations. As previously mentioned, these two violets often came into contact along the borders of rocky streams through hemlock woods. In the sampling procedure, collections were made to either side of this line of contact, and no one collection included plants from both sides. On gradual sandy slopes, toward the streams, this zone of contact was often remarkably narrow and could actually be discerned for some distance ahead by the appearance of the plants alone.

Using only the data from a relatively local study such as this, it is difficult to derive the original or ultimate appearances of the two parents, or the degree to which introgression has already proceeded. By extrapolation from extreme populations the following characters are indicated, however, as typical of *V. cucullata* and *V. septentrionalis*.

V. cucullata in its original condition had nearly reniform leaves, averaging about 66% as long as broad; the mature leaves had 20 or more crenations on each half of the lamina; they had especially deep basal lobes, about a third longer than the shortest length of the lamina. The leaf blades were either completely glabrous or very nearly so; if pubescence was present it was on the upper surface only. Cleistogamous flowers were borne erect and were long and subulate, with especially long sepal auricles.

V. septentrionalis, on the other hand, had narrower, more nearly cordate leaves, about as long as broad, with about 15 crenations per side. The basal lobes were shorter than those of *V. cucullata*. The laminas were pubescent on both surfaces; in addition both the lamina margin and the petioles were quite pubescent. Cleistogenes were prostrate or only slightly ascending; capsules of cleistogamous flowers were short and ovoid, with very short and somewhat spreading sepal auricles.

These are a few of the characters indicated as belonging to (hypothetical) genetically pure populations. Several characters seem to have introgressed very markedly. For example, in this region, *V. cucullata* is nearly always characterized by having the laminas definitely pubescent with very short, stiff, white hairs on the upper surfaces of at least the basal lobes. This condition is not common elsewhere, to judge from published reports. On the other hand, *V. septentrionalis* is less pubescent here than elsewhere, judging from manual descriptions and herbarium specimens seen.

TABLE 2. *Distribution of hybrid index values for the population samples of* V. septentrionalis

Collection number	Index values, frequencies						Total specimens	Mean index
	0	2	4	6	8	10		
23	8	8	5				21	1.71
36	11	10	5				26	1.54
40	4	8	8	1			21	2.57
46	3	8	11	9			31	3.68
47	17	15	5	3			40	1.70
48	24	11	1	4			40	1.25
52	5	11	16	6	1	1	40	3.50
56	9	8	13	6			36	2.89
74	36	17	12	4		1	70	1.66
82	19	12	7	1	1		40	1.65
85	10	6	3	3			22	1.91
86	15	12	7	5	1		40	2.25
96		1	7	5	1		14	4.86
100	4	9	6	5		1	25	3.28
114	1	4	7	3	1	1	17	4.24
122	5	12	17	4	2		40	3.30

The data presented in tables 2 and 3 indicate that genetic barriers between the two entities are very weak. On the other hand, the actual habitats of populations of the two species suggest that they are kept moderately distinct by differences in their edaphic preferences. The two entities may be considered as bordering between edaphic ecotypes and edaphic ecospecies. None of the data here analyzed can demonstrate whether, before the advent of the white man, they were more or less distinct than they now are. However, there is some evidence that man has sufficiently disturbed normal environments to stimulate increased hybridization and introgression. This has been done especially by the release of competition pressure in intermediate forest habitats. Anderson

TABLE 3. *Distribution of hybrid index values for the population samples of* V. cucullata

Collection number	Index values, frequencies						Total specimens	Mean index
	0	2	4	6	8	10		
23			2	1	4	9	16	8.50
41			13	11	14	2	40	6.25
45			5	3	14	18	40	8.25
50			4	11	11	12	38	7.63
55			5	12	11	9	37	7.30
65			3	15	21	1	40	7.00
70			2	5	7	14	28	8.36
73		1	9	14	20	25	69	7.71
83			8	8	14	10	40	7.30
90*	1	3	5	3	2		14	4.29
91**		1	8	8	20	15	52	7.54
99			1	11	14	14	40	8.05
104			2	8	14	16	40	8.20
112			1	7	7	21	36	8.57
116		2	5	12	16	5	40	6.85
121			2	9	16	13	40	8.00

* Population a hybrid swarm between *V. cucullata* and *V. fimbriatula*.

** Adjacent to collection 90.

(1948, 1949) implied that disturbance by man resulted in the *creation* of intermediate habitats. It is well known to field ecologists that intermediate habitats are always present in abundance, especially in ecotonal regions (Gleason, 1939). However, they are normally occupied by well-adapted plants and are not available to hybrid segregates for this reason.

In this study it is difficult, if not impossible, to determine whether we are witnessing a case of initial specific differentiation or of extensive introgression following differentiation in the past. The present situation may result in the evolution of two very distinct entities, or it may result in the genetic swamping of one or both.

As mentioned earlier, hybridization with a number of other stemless blue violets is known for each of the two species discussed here. On or near the Preserve, examples of hybridization of both *V. cucullata* and *V. septentrionalis* with *V. fimbriatula* were seen. A hybrid swarm (collection 90) between *V. cucullata* and *V. fimbriatula* was observed on an open hillside about 5 miles southwest of the Preserve. Two small hybrid swarms between *V. septentrionalis* and *V. fimbriatula* were found on the Preserve. From preliminary observations, it was felt that though some introgression was undoubtedly taking place in these instances, its effects were probably confined to extremely localized situations.

SUMMARY

Results of 32 population sample analyses for *V. cucullata* Ait. and *V. septentrionalis* Greene, taken on or near the Edmund Niles Huyck Preserve in Albany Co., New York, during the summer of 1953, are presented. Hybridization indices were computed for each collection, and it is concluded that not only is hybridization between the two species common in this area, but that introgression has proceeded to a considerable extent in both directions. Many of the characteristics of the two violets have been so nearly obliterated that it is now very difficult to reconstruct their original phenotypes.

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HYBRIDIZATION, HOMOGAMY, AND SYMPATRIC SPECIATION

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Mayr (1947) has presented a strong case against the hypothesis of sympatric speciation in the course of supporting the universal hypothesis of geographic (or at least microgeographic) speciation. In the summary (p. 286) of that paper he made the following statement: "It is unproven and unlikely that reproductive isolation can develop between contiguous populations." Later, Grant (1949), in a discussion of pollination systems as isolating mechanisms, presented a model for sympatric speciation in angiosperms dependent on the known flower constancy of bees. This model indicated the means by which an important mutation in flower color or form might become established within a population, thus resulting in time in a new and well isolated species to which internal isolating mechanisms are unnecessary. In passing, the latter author suggested that hybridization might also provide an example of sympatric speciation. It is the purpose of the present paper to further develop this last hypothesis on the basis of a realistic model suggested by recent studies of hybridization and floral isolation in a group of *Penstemon* species. It is not the purpose of this paper to refute the general conclusions reached by Mayr (l.c.) on the importance of geographic speciation, but merely to indicate that sympatric speciation may be less unlikely than previously considered. The word, species, is here used in its biological sense.

Hybridization in Penstemon

Four species of the section *Peltanthera* Keck of the genus *Penstemon* (Scrophulariaceae) are found in cismontane southern California. One of these, *P. centranthifolius,* belongs to the subsection *Centranthifolii* (Keck, 1937), the other three, according to Keck (l.c.), to the subsection *Spectabiles: PP. grinnellii, spectabilis,* and *clevelandii.* Despite this taxonomic disposition, these last three are quite diverse in external appearances, such as corolla color, shape, and size, and there is every indication that they belong to three different species complexes within which speciation appears to have been predominantly geographic.

Penstemon centranthifolius is a moderately tall (2½ ft.), erect plant with narrowly tubular bright scarlet flowers about an inch long, which are borne on lax pedicels in a semi-pendant manner. It is particularly visited and pollinated by hummingbirds (in southernmost California, mostly *Calypte anna* and *C. costae*), and is a plant of open to moderately open sites on light sandy soils in the coastal sage scrub, chaparral, pinyon-juniper, and yellow pine forest communities.

Penstemon grinnellii is a lower, bushier plant with pale-colored flowers (white to suffused pink or blue) characterized by short, narrow tubes, abruptly expanded, broadly ringent throats and abundant lips, and borne on stout, stiff pedicels. It is a plant of rocky and sandy soils in the yellow pine forest, where it is pollinated by large carpenter bees (*Xylocopa* spp.) whose bodies fit this corolla well.

The other two species are intermediate between these extremes of flower morphology. *Penstemon spectabilis* has flowers whose tubes are about twice the length of those of *P. grinnellii,* are only moderately expanded in the throat, and are mainly of bluish-purple colors. It is a semi-shrubby perennial pioneer in coastal sage scrub and chaparral communities, and is

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pollinated by the larger wasps of the genus *Pseudomasaris,* mainly *Ps. vespoides. Penstemon clevelandii,* the fourth member, is a smaller plant which grows among the rocks of desert-edge canyons in chaparral and pinyon-juniper communities. Its flowers are of much the same shape as those of *P. spectabilis* but are significantly smaller and of reddish-purple and intense magenta colors. The usual pollinators of this species are smaller solitary bees (Anthophoridae) and hummingbirds. All three insect-pollinated species are visited frequently by a small host of other insects, especially smaller leaf-cutter bees (Megachilidae), and *P. centranthifolius* is rarely visited by them as well.

Penstemon centranthifolius occurs sympatrically with all of the other species mentioned here. The hybrid *P. centranthifolius* × *spectabilis* (= *P.* × *parishii*) is fairly common in nature where the parents grow together, *P. centranthifolius* × *grinnellii* (= *P.* × *dubius*) occurs rarely (having been recorded only twice so far as I am aware), and the combination *P. centranthifolius* × *clevelandii* is suggested from slight evidence of introgression in the field (Straw, 1955). At the eastern edge of its range *P. spectabilis* transcends the range of *P. clevelandii,* and the appearance of bearded staminodes in colonies of *P. spectabilis* suggests gene flow into that species from *P. clevelandii. Penstemon grinnellii* and *P. spectabilis,* although normally ecologically separated, occasionally occur together as a result of exceptional range extensions of either species into the territory of the other. There is some inconclusive evidence of introgression of *P. spectabilis* by *P. grinnellii* also (Straw, l.c.). There is no evidence that *PP. clevelandii* and *grinnellii* grow together in nature (both species occur on Santa Rosa Mountain, Riverside County) although it is conceivable that they might rarely do so if *P. grinnellii* establishes itself at a low elevation. The hybrid *P. clevelandii* × *grinnellii* has, however, been successfully made in the garden (Lenz, unpublished). All of these species have the same chromosome numbers ($n = 8$) (Keck, 1951).

Despite the facts that these species occur together in nature and apparently are all interfertile (genetic analysis is still incomplete), there is no evidence that any of the species concerned is in any danger of being swamped. There is no sign of hybrid swarms even in colonies containing very conspicuous hybrids (*PP. centranthifolius, spectabilis,* and their hybrid, *P* × *parishii*). Since these species all have markedly different pollinating mechanisms (hummingbirds, wasps, carpenter bees, solitary bees), it appears that hybridization is limited by their floral isolation, and that hybrids occur only rarely under special circumstances. The combination of very different floral mechanisms and high interspecific fertility relationships places *Penstemon* in a class with some other conspicuous western genera such as *Aquilegia* (Grant, 1952) and *Delphinium* (Epling, 1947; Grant, 1949: 91).

Comparative Morphology of Species and Hybrids

The precise details of hybridization and introgression between these four species are unimportant to the present thesis and will be presented elsewhere (Straw, 1955). It is significant and suggestive, however, that two groups of hybrids, those between *P. centranthifolius* and *PP. grinnellii* and *palmeri* (a desert sister-species of *P. grinnellii* that resembles the latter in floral morphology and has the same pollinators) and between *P. centranthifolius* and *P. spectabilis,* resemble in their floral characters two other species growing in the same region, and that all of these species have distinct pollinating mechanisms.

The most extreme crosses, between *P. centranthifolius* and *PP. grinnellii* (known in nature) and *palmeri* (garden-produced hybrid by Lenz, unpublished), have flower shapes intermediate between the shapes of their parents. The tube of the corolla is longer in the hybrids than in *PP. grinnellii* and *palmeri,* while the throats of the hybrids are moderately expanded, in con-

trast to the almost unexpanded throat of *P. centranthifolius* and the greatly expanded throats of the other two parents. These characteristics in flower shape of the hybrids cause a striking resemblance to another, different species, *Penstemon spectabilis,* which is very common in nature. Such differences as are apparent in color of the corolla and development of a beard on the staminode are of a kind that can be easily modified by selection.

The important point in this situation is that by a single step of hybridization a new type is created that is intermediate between two other flower types in very much the same way that another related species is intermediate. All three of the species (*PP. centranthifolius, grinnellii, spectabilis*) have very different and relatively exclusive pollinators: a hummingbird, a wasp, and a bee (Straw, 1955). By an extension of this fact, it can be reasoned that should this wasp, normal to the intermediate species, happen upon a colony containing the three types it would in all likelihood visit the intermediate type, i.e., the hybrid, to the exclusion of the extreme parental types. By the same token, the normal pollinators of the parents would tend to avoid the hybrid, being excluded mechanically on the one hand and ethologically on the other.

The other important natural hybrid, *Penstemon centranthifolius* × *spectabilis,* resembles in shape, size, and flower color the fourth member of our series, *P. clevelandii.* This resemblance is so close that the hybrid has been and still is occasionally confused with *P. clevelandii* on herbarium sheets (Keck, 1937: 819). Here again a new type is produced by hybridization corresponding to another established species which has its own range of pollinators at least partly different from either of the hybrid's parents.

These hybrids are, furthermore, both viable and at least partly fertile. *Penstemon centranthifolius* × *spectabilis* has a pollen fertility averaging about 50 per cent, as tested by aniline blue in lactophenol, *P. centranthifolius* × *grinnellii* measures 86 per cent fertile, and the F_1 *P. centranthifolius* × *palmeri* has a corresponding fertility of about 35 per cent. Second and subsequent generations, largely backcrosses, are known in two cases, with no evident breakdown so far detected. Such hybrids, even more than the mutant type suggested by Grant (1949), are available through selective pollination for the establishment of new species. There is an indication that precisely this mechanism has been effective, at least rarely, in speciation in the genus *Penstemon.* It is very tempting to postulate such an origin for both *P. spectabilis* and *P. clevelandii,* but so definite a statement must await more evidence, now being sought by genetic techniques.

That sympatric speciation by this method has not been more effective in the production of species is probably due partly to the fact that relatively few such distinct niches are available in nature, the two suggested here having already been filled by good species, and partly to a mechanism identified by Grant (1952) in *Aquilegia.* He showed first, that the intermediate types are relatively less visited by pollinators than the parental extreme types, and second, that the backcross types to either parent are successively submerged into the extreme types by selective pollination favoring the more extreme forms. A study of *Penstemon centranthifolius* × *spectabilis* has shown that it is infrequently visited by any pollinators, but more frequently visited by pollinators of the larger-flowered parent, *P. spectabilis.* Most backcrosses tentatively identified in the field seem to favor that parent, and are probably quickly incorporated into that rather variable species. They may even be the cause of much of its variability.

The gene flow that occurs through introgression or through backcrossing of hybrids to parents is thus, apparently, held to its minimum effectiveness by strong selection pressure due to specific and adaptive floral types and mechanisms. The selective pressure of the pollinators in keeping the species' morphological norms intact and preventing or minimizing the

effects of hybridization seems to be greater than other genetic mechanisms operative here, even in the rather small populations normal to *Penstemon* species.

Homogamy and Sympatric Speciation

Mayr (1947: 272–273) reviewed the evidence for homogamy and concluded that " . . . the evidence in favor of homogamy is virtually nonexistent and that homogamy, where it exists, is not of the type that would lead to the establishment of discontinuities within populations." If, however, we slightly modify the lead sentence in his section on this subject to read, "The concept of homogamy states that within a population the most similar individuals *will preferentially be mated to each other,*" so that it applies better to plants, we find that this is precisely and completely the mechanism by which pollination as an isolating mechanism operates, and is the whole basis for the importance of pollinating systems to the evolution of flowering plants (cf. Grant, 1949, 1950). It is, as well, the mechanism by which swamping is prevented in the cases of *Aquilegia* and *Penstemon* mentioned above.

All that is required for sympatric speciation by means of homogamic selective pollination is that some insect not a normal pollinator of either of the parental species "adopt" a hybrid type and remain constant to it. Considering the many species of bees in many regions, this is by no means either an impossibility or an extreme improbability. Such a bee does not even have to be completely constant for its selective effect to be sufficiently intense to satisfy Hogben's calculations (1946, as quoted by Mayr, 1947: 273). That this is so is shown by the above mentioned facts that despite high inter-fertility of species and occasional inconstancy of pollinators there is no sustained tendency for discrete species of *Aquilegia* or *Penstemon,* at least, to merge into one another. Moreover, homogamy in the present case is in no way dependent on any theories of blending inheritance, upon which it was first postulated.

Summary

A model is suggested in which, through hybridization and pollination by essentially flower-constant insects, sympatric speciation in angiosperms becomes a possible and plausible mechanism. There is evidence that despite frequent hybridizations there is no tendency for distinct species of *Penstemon* to be swamped. The hybrids between some species of *Penstemon* resemble other discrete species, all of which have their own normal range of selective pollinators. Such hybrids may form, or may have formed in the past, new species. The mechanism by which this operates appears to be true homogamy as defined essentially by Mayr (1947). It is thus possible that through homogamy new species may become established without either geographic or strong genetic isolation from their parents.

Acknowledgments

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SUBSPECIATION IN *CLARKIA BILOBA*

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INTRODUCTION

Clarkia biloba (Durand) Nels. & Macbr. (Onagraceae) is an annual species endemic to California in the foothills of the Sierra Nevada and a small area near San Francisco Bay. It varies conspicuously from place to place in external morphology, and particularly in the conformation of the petals. The purpose of this paper is to compare the morphological variation and fertility relationships within this species in relation to the differentiation of geographical races.

Clarkia biloba occurs in colonies that may comprise tens of thousands of individuals, covering several hundred square yards or, more often, several hundred or a few thousand individuals may occur in a correspondingly smaller area. Still smaller colonies are also found and we believe that solitary plants may sometimes occur. The density of most colonies lies between 10 and 100 mature individuals per square yard although seedling densities may be much higher. The colonies may be separated by a few feet or by several miles.

Clarkia biloba is pollinated by insects, particularly bees. The flowers are protandrous. The stigma is held above the anthers, and most, if not all, of the pollen is shed before the stigma of the same flower becomes receptive; consequently, outcrossing is the norm. However, clarkias are self-compatible and self-pollination undoubtedly occurs to whatever extent pollinators visit flowers of different ages on the same plant. Seed set per capsule is usually about 50 and the ordinary plant in the wild will produce 10 to 20 capsules. The seeds have no special means of dissemination and are for the most part dropped near the base of the plant.

MORPHOLOGICAL VARIATION

The chief character by which intercolonial differences can be measured is the petal, in respect of proportions, degree of incision from the apex, and color. Leaf characters can be used to some extent. Taken together, these traits show a fairly regular pattern of variation, graphed in figure 1, which we shall contrast with that of fertility relationships.

The range of morphological variation and the distribution of the species were determined from herbarium collections obtained from the following institutions: California Academy of Sciences, Dudley Herbarium of Stanford University, Missouri Botanical Garden, Gray Herbarium of Harvard University, New York Botanical Garden, Rancho Santa Ana Botanic Garden, Pomona College, University of California, State University of Washington, University of Wyoming, and the United States National Herbarium.

Subsequent to the study of these specimens, collections were made of more than 50 colonies throughout the area of distribution of which 26 were selected for comparison (table 1, figs. 2 and 3). Flower color was determined in the field from fresh material and one flower from each individual was preserved in 70 per cent ethanol for later measurements. In addition, entire plants were carefully pressed and dried, buds for studying meiosis were fixed in acetic-alcohol, and seeds were obtained when available. Progenies from seeds harvested in the wild from 16 populations were grown under uniform cultural conditions in a green-

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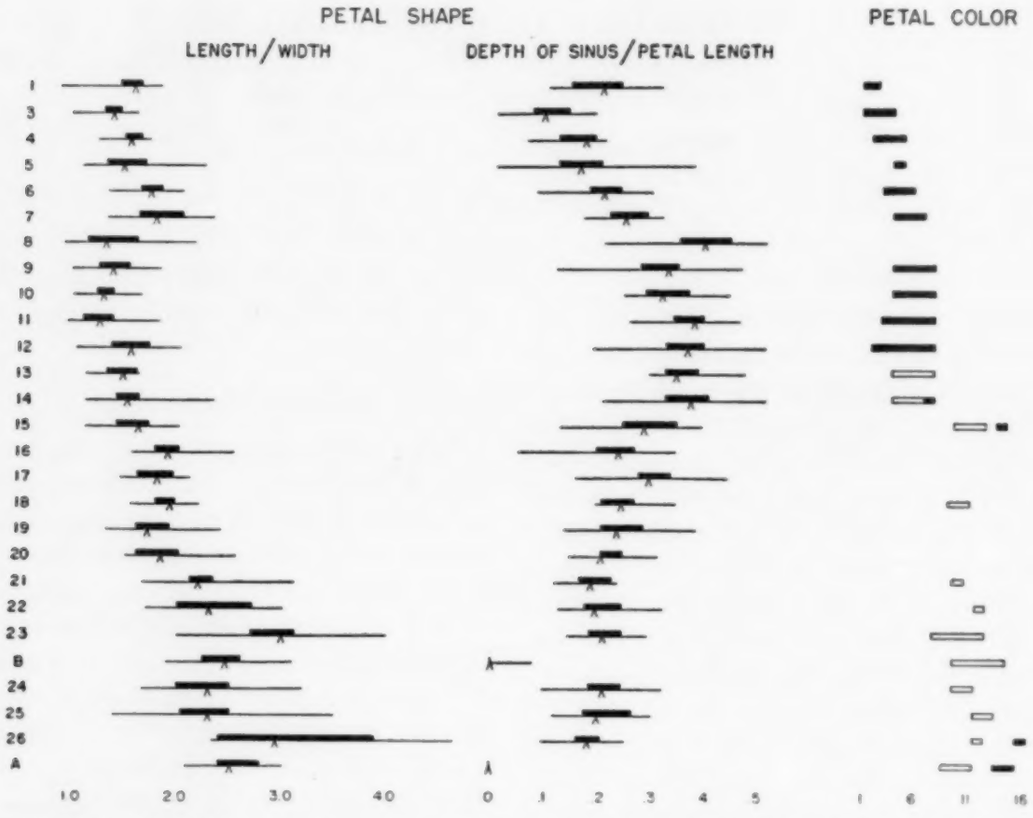

FIG. 1. Variation in petal shape and color in colonies of *Clarkia biloba* (1–26) and *C. lingulata* (A and B). The range of variation in petal shape is indicated by the length of the line, the median is shown by a caret, and the two quartiles are connected by a heavy bar. The range of variation in petal color is indicated by the length of the bar; a solid bar indicates wild plants, an open bar indicates plants grown in a greenhouse. See the text for an explanation of the color scale.

house in Los Angeles. These progenies served as a check on environmental modifications of the field samples and were also used as a source of parents for the hybridization studies.

Petal differences can be expressed by two ratios, that of length to width, and that of the depth of the sinus of the petal to its length. Ratios obtained from petals preserved in 70 per cent ethanol and those from carefully flattened and dried petals from the same population do not differ significantly. Consequently, the ratios obtained from measurement of carefully prepared herbarium collections, such as the two collections of Brandegee listed in table 1, can be compared directly with our field samples. These collections consist of carefully pressed individual flowers selected to indicate, as Brandegee said in a note, "the range of variation in a limited area" and have been included in figure 1 because our samples from the same locality are smaller. Petals of plants grown under green house conditions are proportionally somewhat broader than those from the corresponding wild population. However, the differences between colonies are substantially the same, indicating that the differences in petal proportions between colonies is genetically determined.

Petal differences within and between colonies determined from samples collected in the wild are graphed in figure 1, which is set up as follows. The colonies from Butte County to the Merced River (1–15, table 1) were arranged in their geographical order which generally speaking is linear (fig. 2); the colonies along the south bank of the Merced River (15–26) were arranged from west to east (fig. 3). Two colonies of the closely related *C.*

TABLE 1. *Colonies of* Clarkia biloba *used for morphological, cytological, and hybridization studies*

Colony	Locality	Subspecies	Collection numbers
1.	7 miles from Bangor on the road to Oroville, Butte County	*brandegeae*	394, 822
2.	0.7 mile from Oregon House Post Office, on the road to Stanfield Hill, Yuba County	*brandegeae*	158, 389
3.	1.1 miles south of Newcastle, Placer County	*brandegeae*	819
4.	Highway 49, 3.1 miles south of Pilot Hill, Eldorado County	*brandegeae*	379, 815
5.	Simpson's Ranch, Sweetwater Creek, Eldorado County	*brandegeae*	Brandegee, May 29, 1907
6.	Highway 49, 1.7 miles south of Coloma Post Office, Eldorado County	*brandegeae*	377
7.	3.3 miles north of Plymouth, Amador County	*biloba*	814
8.	Between Martinez and Port Costa, Contra Costa County	*biloba*	442, Brandegee, July 4, 1907
9.	Highway 49, 4.4 miles south of Angels Camp, Calaveras County	*biloba*	811
10.	Highway 49, 2.5 miles south of the Stanislaus River, Tuolumne County	*biloba*	810
11.	Highway 49, 0.2 mile south of junction with Big Oak Flat Road, Tuolumne County	*biloba*	352, 808
12.	Highway 49, 1.5 miles north of the Mariposa-Tuolumne County line, Tuolumne County	*biloba*	807
13.	10 miles west of Coulterville on the road to La Grange, Mariposa County	*biloba*	686
14.	Highway 49, 0.3 mile north of the Merced River, Mariposa County	*biloba*	347, 804
15.	Highway 49, 2 miles south of the Merced River, Mariposa County	*australis*	157, 346, 803
16.	Bear Creek, 1.2 miles south of the Merced River, Mariposa County	*australis*	788
17.	Bear Creek, 0.8 mile south of the Merced River, Mariposa County	*australis*	707, R13
18.	Highway 140, 8.9 miles west of the South Fork of the Merced River, Mariposa County	*australis*	R12
19.	Highway 140, 7.6 miles west of the South Fork of the Merced River, Mariposa County	*australis*	792
20.	Highway 140, 6.1 miles west of the South Fork of the Merced River, Mariposa County	*australis*	R9
21.	Highway 140, 5.8 miles west of the South Fork of the Merced River, Mariposa County	*australis*	631, R8, R108, 705
22.	Highway 140, 2.5 miles west of the South Fork of the Merced River, Mariposa County	*australis*	952, R7, 794
23.	Highway 140, 2.2 miles west of the South Fork of the Merced River, Mariposa County	*australis*	630, 704, R6, R106
24.	Highway 140, 1.5 miles west of the South Fork of the Merced River, Mariposa County	*australis*	628, 701, R4, 950, 798
25.	Highway 140, 1.0 mile west of the South Fork of the Merced River, Mariposa County	*australis*	627, R3, R103, 700, 800
26.	Highway 140, 0.7 mile west of the South Fork of the Merced River, Mariposa County	*australis*	336, 626, R2, R102

lingulata (A and B) were also included for comparison. The range of variation in petal shape within each colony is shown by a horizontal line, the median is shown by a caret and the two quartiles are connected by a heavy bar.

Petal proportions do not vary greatly from north to south, including colony 15

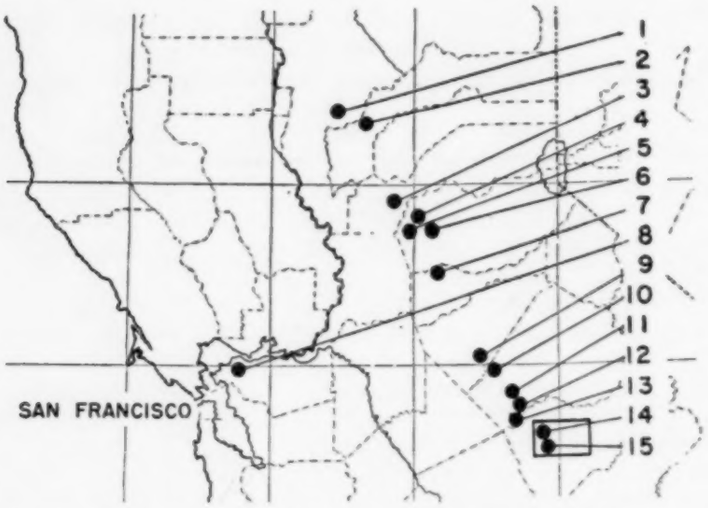

FIG. 2. Distribution of colonies of *Clarkia biloba* (see table 1). For a detailed distribution of colonies along the Merced River see figure 3.

on the south side of the Merced River. Along the south side of the river they become narrower between Hell Hollow (colony 15) and Bear Creek (16 and 17), a distance of about 10 miles. However, because the steep canyon of the river is not easily accessible between these points, no samples have been obtained from this intervening area. We do not know, therefore, whether the change in proportions is gradual or abrupt. From Bear Creek to the limit of distribution of *C. biloba* near the South Fork of the Merced River, a distance of about 10 miles, nine colonies along the river have been examined. Petal proportions are essentially the same for the first four miles (colonies 16–20) but become increasingly more narrow within the next four miles (colonies 21–23). A comparable trend toward narrower petals is found in colonies 25–26 but within a distance of only 0.3 mile. A colony of *C. lingulata* (0.2 mile from colony 23 and 1 mile from colony 24) separates the two clines. A second colony of *C. lingulata* is found 0.5 mile beyond colony 26. It is of interest to note that the colonies of *C. biloba* closest to *C. lingulata* have narrower petals than *C. lingulata* and are the most variable colonies of *C. biloba* with respect to petal proportions.

The pattern of lobing, as indicated by the ratio of the depth of sinus between the lobes to the length of the petal, is different (fig. 1). The populations in the northern part of the range of the species (colonies 1–6) have shallowly lobed petals, some extreme individuals being quite entire. The central and largest part of

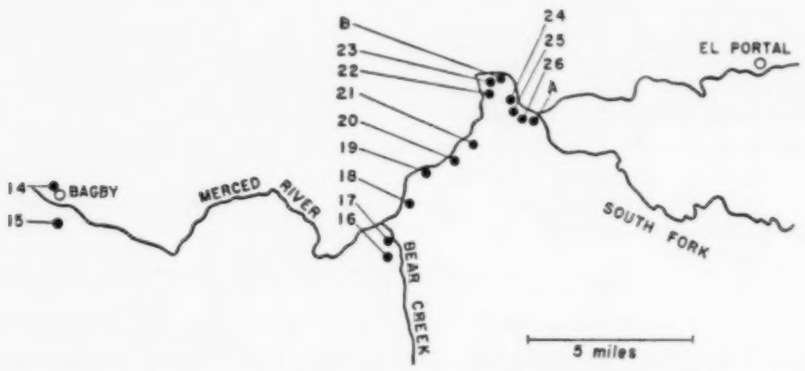

FIG. 3. Distribution of colonies of *Clarkia biloba* (14–26) and *C. lingulata* (A and B) along the Merced River (see table 1). This area enclosed by the rectangle in figure 2.

the area of distribution of the species, represented by colonies 9 through 14, including the disjunct population near San Francisco Bay (colony 8), have conspicuous and deep lobing. A shift toward more shallowly lobed petals is observed between the north side of the Merced River at Bagby (colony 14) and the south side (colony 15), a trend which is continued at Bear Creek (colony 16). From Bear Creek to the limit of distribution of the species about ten miles up the river, the variation in degree of lobing of the petals remains more or less constant, although fluctuating somewhat from one colony to the next. Although the colonies along the south side of the Merced River are characterized by shallowly lobed petals, the extremes are not correlated with the distribution of *C. lingulata,* a species which has entire or very shallowly notched petals. Furthermore, the greatest variation in degree of lobing does not occur in those populations of *C. biloba* that are adjacent to *C. lingulata.*

Flower color has been measured by comparing fresh petals with the colors shown in Mearz and Paul, *A Dictionary of Color,* plates 41 and 49. These plates show combinations of blue to red and purple to red, respectively. Each plate is divided into 144 color combinations with red increasing in 12 steps from left to right (lettered A–L) and with blue or purple increasing in 12 steps from top to bottom (numbered 1–12). Inasmuch as the petals of *C. biloba* range from a reddish-blue to a purplish-red, they can be adequately matched only by using both plates. For purposes of graphic representation we have assigned numbers in sequence following the color gradient represented by the petals of *C. biloba.* Numbers 1 to 6 correspond to the blue-red combinations F5,6 to K5,6 on plate 41; numbers 7 to 16 correspond to the purple-red combinations A8,9,10 to J8,9,10 on plate 49.

The range of flower color for each colony measured is shown in figure 1. Two distinct modes are apparent. Colonies 1 to 14 are definitely bluish in contrast to the bright purplish-red of colonies 15 to 26 and the two colonies of *C. lingulata.* The two color classes are separated geographically, along Highway 49, by the Merced River. The change in color is conspicuously abrupt not only when represented graphically, but also in the field as one crosses from one side of the Merced River to the other at Bagby. The distance between colonies on opposite sides of the river is less than 0.4 mile. On the other hand, populations on opposite sides of the Merced River at the junction with Bear Creek are indistinguishable in color.

These data show clearly that *C. biloba* consists of three fairly well defined morphological races (fig. 4) which replace each other geographically. They have been formally designated as subspecies *brandegeae, biloba,* and *australis* (Lewis

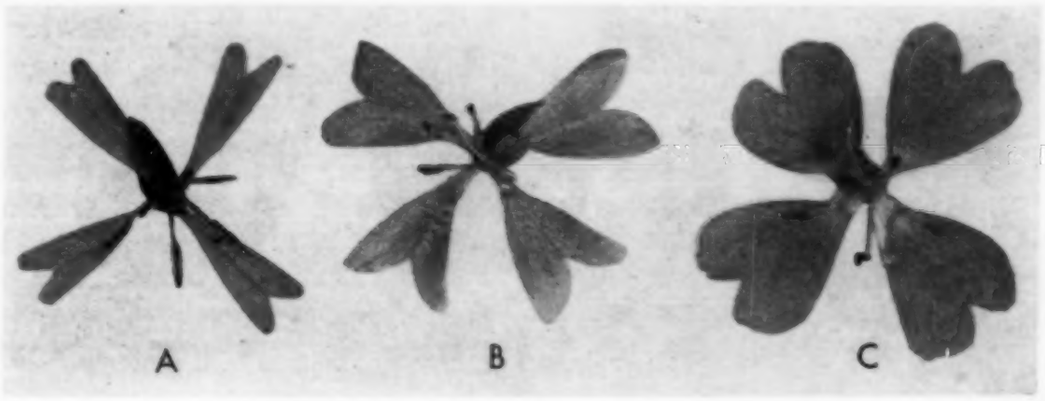

FIG. 4. Flowers of *Clarkia biloba.* A, subsp. *australis* (colony 23); B, subsp. *biloba* (colony 14); C, subsp. *brandegeae* (colony 6).

and Lewis, 1955). The first (colonies 1–6) has broad, shallowly lobed, lavender petals and broad, usually denticulate leaves. The second (colonies 7–14) has deeply lobed, redder petals and narrower and less denticulate leaves. The third, southernmost, subspecies (colonies 15–26) has narrow petals which are only shallowly lobed but bright magenta, and narrow, nearly entire leaves. The transition between the first and second occurs more or less abruptly near Coloma; between the second and third less clearly somewhere between Bagby and Bear Creek, depending upon the characters used. Flower color, but not other characters, changes abruptly at the Merced River.

Intercolonial Hybrids

Crosses were made involving 50 combinations of 13 different colonies. Five colonies (1, 2, 3, 4, and 6) represented subsp. *brandegeae,* three colonies (8, 9, and 13) represented subsp. *biloba,* and seven colonies (15, 18, 21, 23, 24, 25, and 26) represented subsp. *australis.*

Seed set was high in all of the crosses and hybrids were easily obtained. In all instances the progenies were morphologically intermediate. Fertility of the hybrids was estimated by the per cent of visibly good pollen, and the number of good seeds after selfing and backcrossing. Pollen from unopened anthers, just before anthesis, was stained with cotton blue in lactophenol. For most plants 100 pollen grains from each of three buds were scored, making a total of 300 pollen grains for each plant. The fertility of the parents was used as the norm for estimating the fertility of the hybrids. The results were as follows. All of the hybrids between colonies of the same subspecies were fully fertile. All of the hybrids between colonies of subsp. *australis* and subsp. *biloba* were also fully fertile. On the other hand, the hybrids between subsp. *brandegeae* and either of the other subspecies showed a marked reduction in fertility. Visibly good pollen varied from 31 to 63 per cent per individual in the latter, with a mean near 50 per cent (with subsp. *biloba,* mean of 50.5 per cent; subsp. *australis,* mean of 47 per cent). Seed set was correspondingly reduced. This consistent reduction in fertility in hybrids involving subsp. *brandegeae* suggests that a single segregating factor, perhaps a small reciprocal translocation may be involved. Test of the validity of this hypothesis are in progress.

Cytological Observations

The haploid chromosome number of *C. biloba* is 8 (Håkansson, 1941). We have observed meiosis in microsporocytes of 114 individuals derived from 17 colonies, representative of the entire morphological and geographical range of the species (table 2). All of the plants examined had 8 pairs of chromosomes with no evidence of chromosomal rearrangement. Two chromosomes were occasionally found to be unpaired at meiotic first metaphase, but the position of the chromosomes suggested precocious separation rather than failure of pairing. However, two plants, both from colony 22, each had one microsporocyte with groups of 7 and 9 chromosomes at the poles in early telophase, rather than the expected 8.

Microsporocytes of 12 intercolonial hybrids involving combinations between all of the subspecies were also examined.

Table 2. *Colonies of* Clarkia biloba *examined cytologically*

Subspecies	*brandegeae*				*biloba*				*australis*								
Colony	1	2	3	4	7	8	11	13	15	18	19	21	22	23	24	25	26
Number of plants examined	2	3	1	6	6	1	17	1	11	5	2	1	23	3	16	13	3

Results: All plants had 16 chromosomes which regularly formed 8 pairs at meiotic first metaphase.

TABLE 3. *Maximum chromosome association in microsporocytes of intercolonial hybrids of* Clarkia biloba

Subspecies	*aust.* ×*aust.*		*biloba* ×*biloba*	*brand.* ×*brand.*	*aust.* ×*biloba*	*aust.* ×*brand.*			*biloba* ×*brand.*	
Colonies	15×24	15×26	8×13	1×4	23×13	26×4	15×4	15×2	13×4	13×6
Number of plants	2	1	1	1	1	1	2	1	1	1
Chromosome association	8_{II}	8_{II}	16_{I}	$6_{II}1_{IV}$	8_{II}	8_{II}	8_{II}	8_{II}	8_{II}	8_{II}

Only two exceptions to the formation of 8 pairs of chromosomes were found. A hybrid between two colonies of subsp. *brandegeae* (1 and 4) was heterozygous for a large translocation, indicated by the regular formation of a ring of four chromosomes. However, alternate disjunction of the chromosomes in this ring was regular and fertility was therefore not impaired. Second, a hybrid between two colonies of subsp. *biloba* (13 and 8) showed complete asynapsis. However, this may have been due to environmental factors (Lewis, 1953). The hybrids between subspecies regularly formed 8 pairs of chromosomes, which suggests that their chromosomes do not differ by gross rearrangements; nevertheless, the reduced fertility of inter-subspecific hybrids involving subsp. *brandegeae,* as well as differences in chromosome association in hybrids between all three subspecies with the closely related species, *C. lingulata* (Lewis and Roberts, in press) does suggest structural differences of some sort in subsp. *brandegeae.* The hybrid between subsp. *australis,* with a genome of 8 chromosomes and *C. lingulata* with 9, usually has 4 pairs, a ring of 4 and a chain of 5 chromosomes at meiotic first metaphase (Lewis, 1954). The same is also true of the hybrid between *C. lingulata* and subsp. *biloba.* On the other hand, the hybrid between *C. lingulata* and subsp. *brandegeae* usually has 5 pairs, a ring of 4 and a chain of 3, although a chain of 5 is occasionally found, suggesting, thereby, a structural difference between subsp. *brandegeae* and the other two subspecies, which reduces chiasma formation. The chromosomes involved have submedian centromeres and in the parents normally form a chiasma in each arm. One may therefore suspect a translocation which would so reduce chiasma formation between the interchanged arms in the heterozygote that only pairs rather than a ring would usually be formed. Such a translocation would account for the seeming regularity of meiosis in hybrids that are only 50 per cent fertile, inasmuch as the heterozygous pairs would dissociate independently, resulting in duplications and deficiencies in half of the meiotic products. Such a rearrangement would also have a different potential for chromosome association in other hybrids, such as the observed difference between a chain of 3 and a chain of 5 chromosomes in hybrids between *C. biloba* and *C. lingulata.* If these inferences are valid, one would expect an increased number of rod, rather than ring, bivalents in the hybrids between subsp. *brandegeae* and the other subspecies, and perhaps an occasional translocation ring of four. Both phenomena may perhaps occur, but unfortunately these hybrids tend to have sticky chromosomes which makes difficult a precise determination of chiasmata and multivalents.

Thus, it would appear that the reproductive barrier which exists between subsp. *brandegeae* and the other subspecies is probably associated with chromosomal rearrangements of some sort.

RELATIONSHIP TO OTHER SPECIES

A comparison of the external morphology of *C. biloba* with its close relatives shows the interesting fact that the two extreme races, subsp. *australis* and subsp. *brandegeae,* are more similar to other spe-

cies, *C. lingulata* and *C. dudleyana* respectively, than they are to each other (Lewis and Lewis, 1955).

Subsp. *australis* and *C. lingulata* grow in adjacent colonies (fig. 3) and can be distinguished only by the lobed petals of the former and chromosome number (n = 8 and 9, respectively). Hybrids between them are highly sterile but a few individuals can be obtained by backcrossing (Lewis and Roberts, in press). Meiosis in the hybrid indicates that the two species have structurally comparable genomes except for one translocation, at least one inversion, and an extra chromosome in the genome of *C. lingulata*. This additional chromosome is homologous to parts of two other chromosomes in the genome (Lewis, 1954).

Subsp. *brandegeae* closely resembles *C. dudleyana* and differs from it chiefly in having lobed and more or less uniformly colored petals, and in chromosome number (n = 8 and 9, respectively). The petals of *C. dudleyana* are entire and usually streaked with white. But extreme variants of subsp. *brandegeae* have entire petals and occasional individuals of *C. dudleyana* have a distinct notch at the apex of the petal. Furthermore, some individuals of *C. dudleyana*, particularly in the southern part of its area of distribution, have more or less uniformly colored petals comparable to those of subsp. *brandegeae*. Consequently, individuals are found which on the basis of external morphology are difficult, if not impossible, to assign to the appropriate species. Nevertheless, subsp. *brandegeae* and *C. dudleyana* are separated by a distance of 75 miles and we have been unable to obtain a mature hybrid between *C. dudleyana* and any of the subspecies of *C. biloba* despite numerous attempts with different individuals from different populations.

Discussion

The colonies of *C. biloba* are more or less evenly distributed along the Sierra Nevada foothills from north to south and along the south bank of the Merced River from west to east. Three subspecies which replace one another geographically are represented, but adjacent subspecies are no farther separated from each other than are the colonies within them. This pattern of geographical subspeciation is comparable to that described for many organisms and may be indicative of adaptive ecological differentiation. However, the sites occupied by the subspecies of *C. biloba* are similar and the ecological differences between them are those associated with the general climatic gradient from north to south. But the morphological differences between the subspecies are more or less abrupt rather than gradual and do not therefore appear to be closely correlated with differences of environment. In this respect the pattern of subspeciation in *C. biloba* differs from that described for other Californian plant species (e.g. *Layia platyglossa*, Clausen, 1951) in which the morphological modes are obviously associated with differences in habitat. Thus, subspeciation in *C. biloba* appears to be the result of barriers to gene exchange other than those imposed by ecological differentiation. These barriers are of two sorts. First, and perhaps more important, is that of distance as a result of the colonial habit; and second, an internal barrier which greatly reduces fertility.

The fertility of their hybrids shows no internal barriers to gene exchange between subsp. *biloba*, the central race, and subsp. *australis*, the southern race. Thus, the difference in flower color between colonies of these subspecies, separated by less than a mile across the Merced River, is perhaps maintained by the barrier of the river canyon. This inference is supported by the absence of effective gene exchange over a period of 5 years between colonies of *C. xantiana* which are separated by no greater barrier than 50 feet (Lewis, 1953). Comparable barriers involving short distances are known in *Delphinium hanseni* (Epling and Lewis, 1952) and are doubtlessly effective in other colonial species. The spacing of colonies may also be a

factor in the chance fixation of genetic deviants, particularly when new colonies may be established from a few seeds or possibly only one.

The reduction in fertility of the hybrids between subsp. *brandegeae* and the other two races may be due to a single structural rearrangement which results in the elimination of half of the products of meiosis, as we have pointed out. But this barrier would probably be supplementary to that imposed by the spacing of colonies which may itself have been instrumental in establishing whatever factor is operating to reduce fertility. The heterozygote would be expected to be at a selective disadvantage since it greatly reduces fertility. Consequently, the establishment of a colony and eventually a race homozygous for such a factor would depend either upon a selective advantage of the altered homozygote or upon chance fixation in a small population. Experiments now in progress may indicate the mechanism. However, in view of the constant differences which frequently distinguish adjacent colonies in *Clarkia* (Lewis, 1953), we regard the second explanation as more likely.

The geographical variation shown in figure 1 may have come from gene mutation within the species or interspecific hybridization. Interspecific hybridization may also be the cause of the two conspicuous clines in petal proportion (fig. 1) which are found in subsp. *australis* adjacent to the two known colonies of *C. lingulata* as well as the greater variation of the colonies closest to them. Furthermore, subsp. *australis* is morphologically and geographically intermediate between subsp. *biloba* and *C. lingulata,* and thus may represent the hybrid product between them. On the other hand, other observations suggest that the pattern of variation is not the result of interspecific hybridization. For example, the colonies of subsp. *australis* nearest those of *C. lingulata* have even narrower petals than that species. This might be explained as transgressive segregation for petal proportions in hybrid derivatives, but our data from first and second backcross progenies do not support this view. Furthermore, the genomes of subsp. *australis* are indistinguishable from those of subsp. *biloba* but differ from those of *C. lingulata* by a translocation, at least one inversion and the addition of an entire chromosome (Lewis, 1954). These differences segregate independently in the hybrids and many of the individuals in the second backcross progenies are heterozygous for at least one of these chromosomal differences. One might expect, therefore, to find occasional heterozygotes in the wild populations if hybrids were formed, but none has been found in 77 individuals of subsp. *australis* nor in a comparable number of *C. lingulata*. The most likely explanation of their similarities is that *C. lingulata* arose from subsp. *australis*. This explanation is further developed elsewhere (Lewis and Roberts, in press).

Subsp. *brandegeae* is morphologically intermediate between subsp. *biloba* and *C. dudleyana* and is sometimes scarcely distinguishable from the latter without counting the chromosomes. This intermediacy suggests the possibility of a hybrid origin of subsp. *brandegeae*. However, we have not been able to obtain the hybrid between *C. dudleyana* and subsp. *biloba* despite numerous attempts nor have we found the hybrids in the wild, although the two taxa grow together in mixed colonies. The present distribution, in fact, precludes hybridization in the immediate past as an explanation inasmuch as subsp. *brandegeae* and *C. dudleyana* are separated by a distance of at least 75 miles.

Considering all of the evidence together, we have found no reason to believe that the geographical variation in *C. biloba* is attributable to interspecific hybridization.

Summary

Clarkia biloba varies conspicuously in the shape and color of the petals. The traits that were measured have different patterns of variations, but taken together, three fairly well defined morphological races, designated as subspecies, can be

recognized. These subspecies replace one another geographically along the foothills of the Sierra Nevada. The two southern subspecies are completely interfertile but fertility is reduced to about 50 per cent in hybrids between the northernmost subspecies and the other two. This reduction in fertility is probably due to structural rearrangement of the chromosomes. The more or less abrupt transition between the subspecies is not correlated with discernible ecological differences; consequently, subspeciation has probably resulted from barriers to gene exchange due to the spacing of the colonies, supplemented in the northern subspecies by a factor which reduces fertility in heterozygotes. The pattern of variation within the species has probably not been modified by interspecific hybridization.

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INTERSPECIFIC HYBRIDIZATION IN *BUFO WOODHOUSEI* AND *BUFO VALLICEPS*

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INTRODUCTION

The toad species *Bufo woodhousei* and *Bufo valliceps* are considered by taxonomists to be distinct sympatric species. *Bufo woodhousei* is principally an inhabitant of the Great Plains although its range extends from the northern states of Washington, Montana, and North Dakota south into Mexico. The Mexican toad (*Bufo valliceps*) occurs in the lowlands of Mexico south into Costa Rica. In the United States, this species ranges from the Pecos River in western Texas east through the central, southern, and eastern parts of the state into Louisiana. The ranges of the two species overlap in the United States throughout approximately the eastern half of Texas. Both species breed in a variety of habitats in areas of both temporary and permanent water. In external morphology the two forms are effectively distinct, and the calls are different.

The dynamic species concept accepted by most modern students of evolution requires a definition of the species as a population that is reproductively isolated from other populations. In this particular case we are dealing with two distantly related, sympatric species which are apparently able to retain their distinctness in spite of a broad overlap in their ranges. Such a situation offers an excellent opportunity to study the possible isolation mechanisms which are presumably in effect, and at the same time to search for evidence of interspecific hybridization. The present report, dealing only with the work on hybridization, is a part of a more comprehensive study (in manuscript) concerned with other probable isolation mechanisms, and with the general activity of several marked populations of toads during four consecutive breeding seasons.

REGION STUDIED

The region studied lies within the environs of the city of Austin, Travis County, in south-central Texas. Field observations for this report extending over a period of four years, 1950 through 1953, were carried out in one general area of the city. An area in southeastern Austin, where dirt ponds of the U. S. Federal Fish Hatchery are in close proximity to the Colorado River.

The hatchery is located on what was once a part of the flood-plain of the Colorado River. The area consists of approximately 20 man-made dirt ponds separated from each other by dirt dikes formed from the excavations for the ponds themselves. The tops of the dikes are level and wide and slope gradually on either side to the water's edge. Grasses form the only vegetative covering of the tops and sides of the dikes. The ponds are rectangular and vary in size from 75 feet to 200 feet in length and from 50 feet to 100 feet in width. Water from the nearby river is pumped into the ponds to maintain a constant water level.

During the overlap of breeding times male individuals of both toad species are found along the edges of the various ponds and occasionally on the tops and sides of the dikes. No preference for particular ponds has been observed for individuals of either species. Toad activity varies from pond to pond during the breeding season.

METHODS

Experimental cross-matings and control matings in the laboratory were carried out

at room temperature in enamel pans containing pond water and waterweed (*Elodea canadensis*). The white enamel pans measured nine inches wide, 15 inches long, and two and one-quarter inches deep. The separate clasping pairs placed in these pans were prevented from escape by a seven inch high wooden framework covered with one-quarter inch hardware cloth which was made to fit around the inside of the rectangular pans.

The zygotes produced from the crossmatings and controls were distributed in enamel pans with enough waterweed for attachment of the egg strings. Developing larvae were raised through metamorphosis in these pans (30 to a pan) on a diet of boiled lettuce leaves. After metamorphosis the young toads were fed on a diet of fruit flies (*Drosophila*) until old enough to accept larger insects.

Artificially induced backcrosses were carried out in the laboratory using the procedure described by Rugh (1948, p. 104). The testes of the hybrid and control were removed and mascerated in small amounts of pond water. The sperm suspensions were divided between several petri dishes. Eggs were stripped directly into each sperm suspension. The eggs from a single individual were divided between the sperm suspension from the hybrid and from the controls. Separate forceps were used to extract the testes and to make the sperm suspension in order to rule out the possibility of contamination.

Frequency of Cross-Pairing in Nature

The toads (*Bufo*) are representative of animals that form breeding swarms. At some times of the year, large mixed choruses of *woodhousei* and *valliceps* toads occur in the dirt ponds of the Federal Fish Hatchery. Crossmatings (female *woodhousei* with male *valliceps* and female *valliceps* with male *woodhousei*) have been observed.

On nine observation nights representing two breeding seasons (1951, 1952), counts were made of the number of toads of both species found breeding in specific hatchery ponds. Toad activity varied from pond to pond on the different nights of observation. Table I shows the total number and sex of both toad species present, the number of normal matings, the number of cross-matings, and the ratio of cross pairs to the total number of clasping pairs, for each observation night.

A total of 1,710 toads was counted (1,361 *woodhousei;* 349 *valliceps*) representing 1,269 *woodhousei* males, and 92 *woodhousei* females; 326 *valliceps* males,

Table I. *The number of* woodhousei *and* valliceps *toads present in separate dirt ponds located at the Federal Fish Hatchery in southeastern Austin*

The counts show the total number of each species and sex of toads observed, the number of species pairings, the number of cross-pairings, and the percentage of cross-pairs in the total number of clasping pairs recorded on nine observation nights.

Date	No. of ponds	Total *woodhousei* ♂♂	Total *woodhousei* ♀♀	Total *valliceps* ♂♂	Total *valliceps* ♀♀	*woodhousei* ♀ pair	*woodhousei* ♀ X-pair	*valliceps* ♀ pair	*valliceps* ♀ X-pair	% X-pairs
3–6–51	3	238	10	1	0	9	1	0	0	10
3–7–51	2	238	7	2	0	7	0	0	0	0
4–10–51	1	34	3	10	1	3	0	0	1	25
4–18–51	1	5	0	9	1	0	0	1	0	0
5–17–51	6	189	12	172	7	8	3	7	0	17
5–18–51	4	34	0	59	4	0	0	4	0	0
3–30–52	5	477	57	16	2	55	3	1	1	7
3–31–52	2	37	0	30	7	0	0	6	0	0
5–6–52	1	17	3	27	1	3	0	1	0	0
Totals	25	1,269	92	326	23	85	7	20	2	

and 23 *valliceps* females. Of the 1,710 toads counted, 105 were taken in normal species amplexus (85 pairs *woodhousei;* 20 pairs *valliceps*), and nine were cross-matings (seven pairs involving female *woodhousei* with male *valliceps* toads, and two pairs of female *valliceps* with male *woodhousei* toads). The nine cross-clasping pairs represent eight per cent of the total number of clasping pairs recorded.

There would appear to be no mating preference, at least on the part of the male toads, during the breeding season. The calls of the two species are different and are presumably involved in species recognition. In large mixed breeding populations of *woodhousei* and *valliceps* toads, a female toad responding to the sex call of her own species or to a physiological response to return to a breeding area to spawn, might approach and be clasped by a sexually excited male of another species. The number of cross-matings occurring in this manner could theoretically affect the amount of gene exchange between the sympatric species if difference in call was the only isolation mechanism. The high frequency (8 per cent) of cross-pairs in this population would suggest that a relatively high degree of genetic exchange is possible in the hatchery breeding ponds unless there is a genetic block to such exchange.

The role of a genetic block in restricting gene exchange between thse sympatric species will be discussed in a later section of this paper. Additional information concerning the frequency of natural hybridization can be obtained by searching for apparent hybrids in collections of field-caught toads.

Frequency of Occurrence of Natural Hybrids

Cross-mating has been observed at only one locality in the Austin area; the Federal Fish Hatchery in southeastern Austin. Six sample collections of *woodhousei* and *valliceps* toads were made in 1951, and 1952 at this locality. Toads were preserved in 10 per cent formalin. An intensive search has been carried on, as well, to identify individual toads having "unusual" calls, or individuals having external characters intermediate between the two species. It should be pointed out here that no difficulty is encountered in distinguishing adult *woodhousei* toads from *valliceps* toads in nature. The more obvious visible morphological differences between the two species are found in the body pattern and in the development and arrangement of the bony crests on the top of the head.

Anderson (1949) offers a number of special techniques to estimate relationships in potentially hybridizing natural populations. One of these techniques, the scatter diagram, has been used here.

The individual toads from each of the collections made at the hatchery ponds were measured in millimeters for greatest parotoid length, greatest width of tympanum, greatest width of inner metatarsal tubercle, and depth of the valley formed between the interorbital crests (taken at the mid-point of a line between the eyes). The ratios of parotoid over tympanum, and tubercle over crest were used to produce a scatter diagram. The vertical coordinate for each diagram represents the ratio of parotoid over tympanum. The horizontal coordinate represents the ratio of tubercle over depth between interorbital crests. The toad characters chosen for measurement are presumed to be independent of each other, or of any common factor. The ratios clearly separate the two species when they are plotted on a scatter diagram.

Both species are represented on the diagrams by solid black circles. *Bufo woodhousei* has longer parotoids, more shallow crests, and a much wider tubercle (spade), than does *B. valliceps*. For this reason the ratio of tubercle over crest, and the ratio of parotoid over tympanum are consistently larger than the comparable ratios for *valliceps*. The specimens of *woodhousei* consequently fall in the upper right quadrant on the scatter diagram. Because of their smaller ratios, specimens

of *valliceps* fall in the lower left quadrant. First generation hybrids would be expected to fall in a zone between the parental forms if their morphological characters were intermediate between those of the parent species. Introgressive hybridization, if present, would be expected to show on a scatter diagram as a general blending of the morphological characters of one species into the other.

The six random sample collections from the Federal Fish Hatchery (figs. 1–6) represent a total of 297 toads (208 *woodhousei,* 89 *valliceps*). Each scatter diagram represents one collection. Six probable natural hybrids which were collected on nights *other* than those on which random samples were taken have been plotted on the scatter diagram of 62 *woodhousei* toads (fig. 3) made on February 28, 1952. The ratios of three laboratory hybrids from the cross of female *woodhousei* with male *valliceps* have also been included on this scatter diagram.

From the collections made at the hatchery ponds and from individuals collected as "intermediates" on various observation nights at the hatchery, a total of 11 probable natural hybrids has been recognized. Six probable hybrids out of the 11 recognized, were collected on *separate* observation nights over a three year period. The remaining five probable natural hybrids (one per cent) were recognized as a part of the six random toad collections made at the hatchery. The ratios of the probable hybrids recognized and the ratios of the laboratory-raised hybrids when produced as a scatter diagram, fall in an intermediate area between the parent species.

As already mentioned, 114 clasping

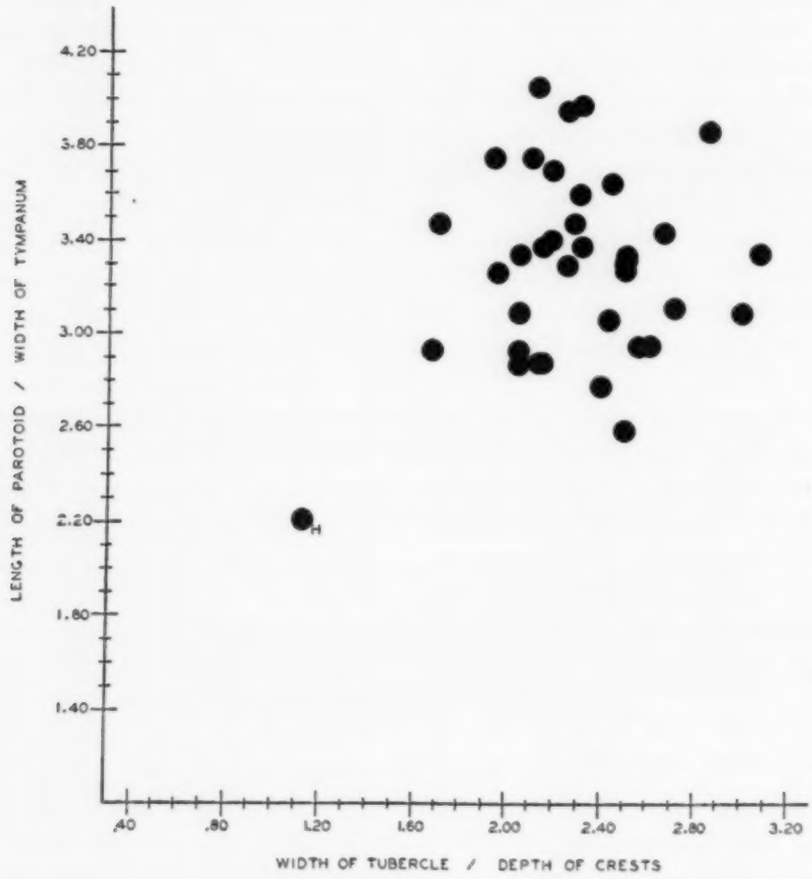

FIG. 1. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 37 toads (36 *woodhousei,* and one probable natural hybrid) collected at the U. S. Federal Fish Hatchery in southeastern Austin on February 19, 1952. The letter H is used to designate the probable natural hybrid.

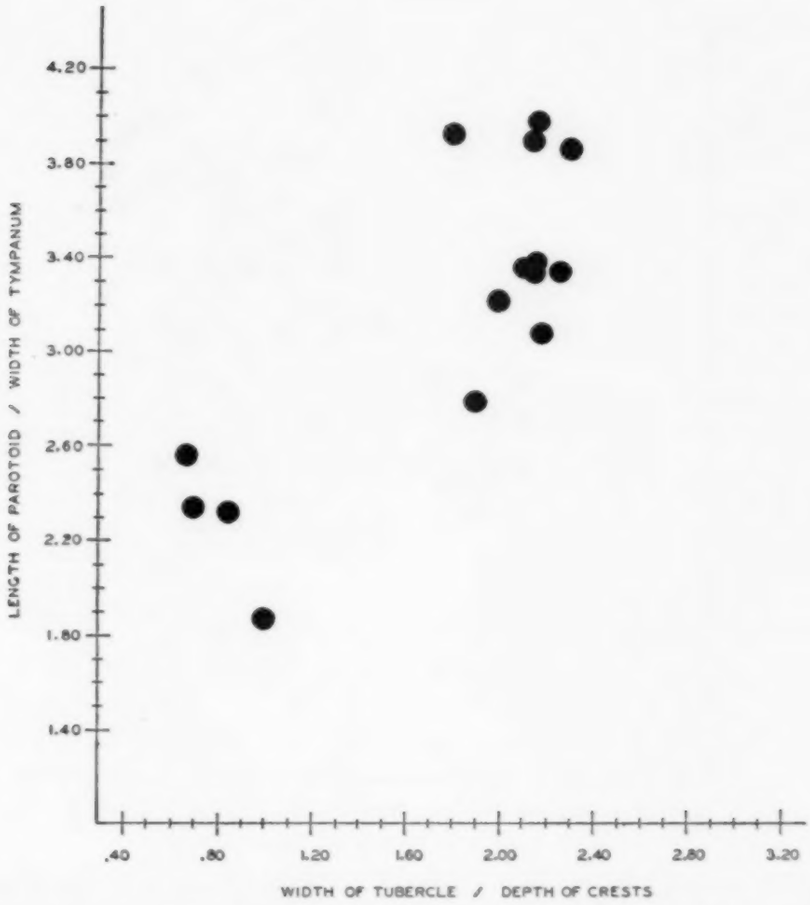

FIG. 2. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 15 toads (11 *woodhousei*, and four *valliceps*) collected at the U. S. Federal Fish Hatchery in southeastern Austin on May 15, 1951.

pairs were recorded in the hatchery ponds on nine observation nights covering two breeding seasons. Of these, nine pairs (eight per cent) were cross-matings. Seven of the nine cross-matings (six per cent) involved *woodhousei* females with *valliceps* males. This cross-pairing in the laboratory, as will be discussed later, is known to produce viable zygotes at least some of which pass through the larval stages and metamorphose. Zygotes from the reciprocal cross are inviable. None survive beyond the late neurula stages in development.

The high frequency of cross-matings producing viable zygotes (six per cent) in the hatchery population, when compared with the low percentage (one per cent) of probable hybrids recognized in six random sample collections, would suggest that selection is working against the hybrid toads.

The discovery of apparent natural hybrids (presumably F1) is evidence additional to the discovery of the cross-mated pairs that natural hybridization does occur in some places between these two species.

LABORATORY SPECIES CROSSES

Crosses were made in the laboratory between the two species in the spring months of both 1951 and 1952. Artificial insemination was not used. The toads were collected in the field during the breeding season, and usually toads were selected for use in the laboratory experiments that were observed cross-mating in nature. No difficulty was encountered however in getting the male of either species to clasp any of the females. No species preference was discernible. The fer-

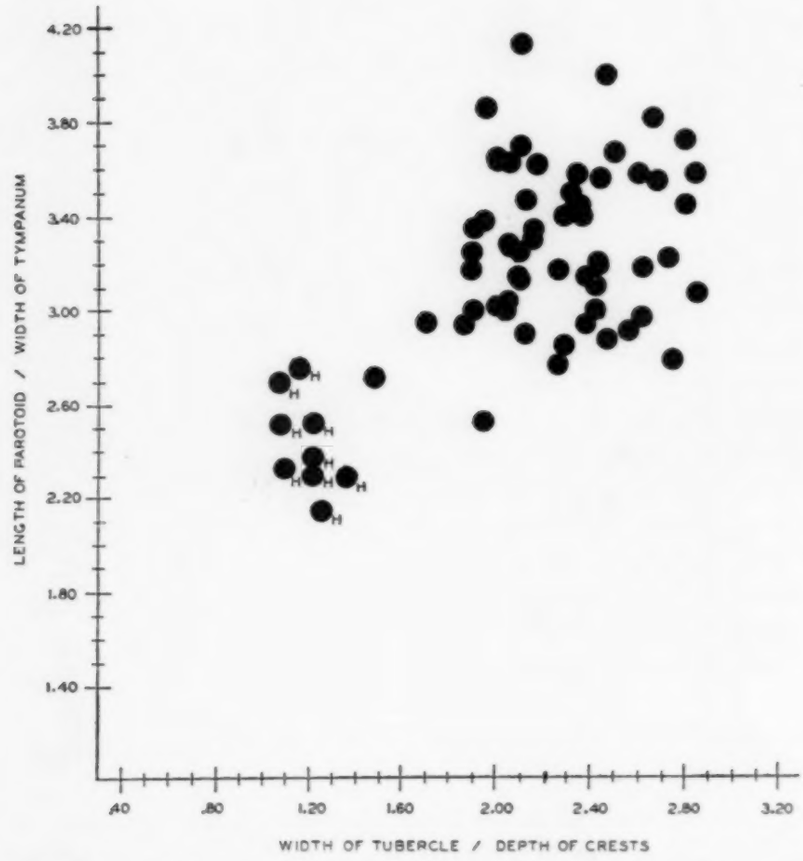

FIG. 3. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 62 *woodhousei* toads collected at the U. S. Federal Fish Hatchery on February 28, 1952. Six probable natural hybrids which were collected at this locality on nights *other* than those on which random samples were taken, and three laboratory raised hybrids are represented on the diagram by the letter H.

tilized eggs from each pair were kept in separate, large, enamel pans containing pond water at room temperature. Samples of embryos were taken at various stages of development. The stages in embryonic development were compared with photographs of the complete stages in the larval development of the leopard frog, *Rana pipiens* (Rugh, 1948, Stages 1–25). Possible abnormalities in the embryos were compared with photographs of abnormalities in *R. pipiens* (Rugh, 1948, Numbers 1–95).

Valliceps Female with *woodhousei* Male: Fourteen cross-pairings (*valliceps* female with *woodhousei* male) were carried out in the laboratory in 1951. In none of these crosses were the toads ones that were first observed cross-mating in nature, and all were paired after being brought into the laboratory. In two of the attempted crosses the females failed to ovulate. Twelve of the cross-paired females laid eggs, and cell division began in at least some of each batch of eggs. None of the embryos, however, survived beyond the late neurula stages of development.

In 1952, two female *valliceps* and two male *woodhousei* were collected at the Federal Fish Hatchery and paired under laboratory conditions, at 11:30 P.M. on March 31. The two pairs were placed in separate enamel pans with wire covers. The zygotes produced were undergoing early cleavage when examined at 8:00 o'clock the following morning. Ovulation is estimated to have occurred during the early morning hours (5:00–6:00) of April 1. The majority of the eggs examined from these cross-pairings had been

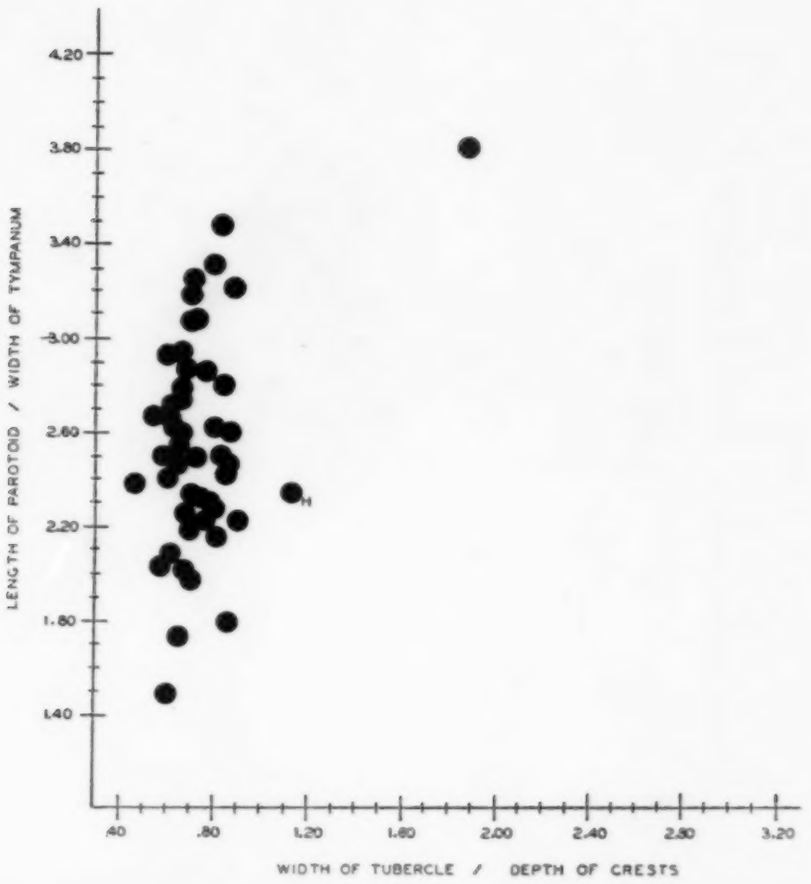

FIG. 4. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 53 toads (51 *valliceps*, one *woodhousei*, and one probable natural hybrid) collected at the U. S. Federal Fish Hatchery on April 29, 1952. The letter H is used to designate the probable natural hybrid.

fertilized. Samples were taken on April 2, 3, 4 and preserved in five per cent formalin. The samples taken on April 2 (approximately 24 hours from the time of ovulation) were found to be in the process of gastrulation (early to medium-sized yolk-plug stages). On April 3 (approximately 48 hours from the time of ovulation) development had proceeded to late gastrulation and early neurula stages (Rugh, 1948, Stages 12 and 16). A probable abnormality was observed in the samples taken at this time. A persistent yolk plug was found in several of the early neurulae. This probable abnormality closely approximates an abnormality (Number 16) noted by Rugh (1948) in the development of *Rana pipiens*. Development presumably came to an end approximately 72 hours after ovulation. Samples examined at this time (April 4) were found to have developed as far as the tail bud stage (Rugh, Stage 17), which corresponds to a stage in embryonic development between the closed neural tube and earliest tadpole.

In the laboratory in 1953, as a further check on the results obtained from this cross, *valliceps* eggs were artificially inseminated with *woodhousei* sperm. Approximately 50 per cent of the eggs used in this cross were fertilized. All development ceased in the late neurula stages. The results of these experiments indicate that the two species could not exchange genes through the cross-mating of female *valliceps* with male woodhousei.

Woodhousei Female with *valliceps* Male: Several crosses of female *woodhousei* with *valliceps* male were effectively

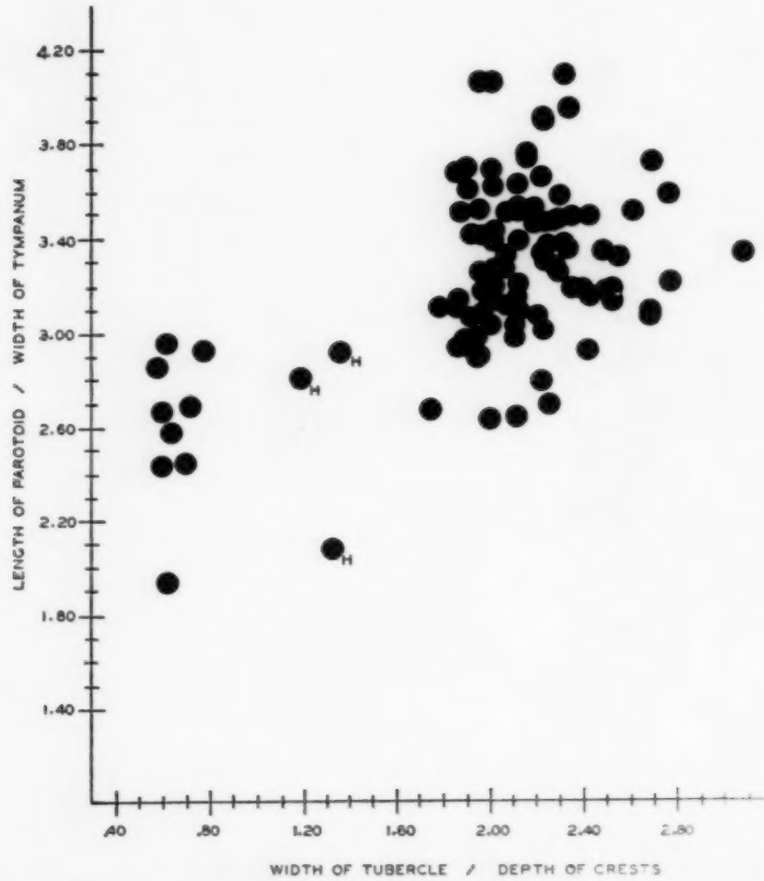

FIG. 5. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 106 toads (94 *woodhousei*, nine *valliceps*, and three probable natural hybrids) collected at the U. S. Federal Fish Hatchery on March 30, 1952. The letter H is used to designate the probable hybrid animals.

carried out in the Spring of 1951. No abnormalities were apparent at any stage in the development of the hybrid embryos. The tadpoles produced went through metamorphosis, but attempts to raise them in the laboratory were unsuccessful. The young toads reached a size of approximately 20 mm. (snout-vent) and died. The cause of death was not determined, but success in raising hybrids from this cross-mating the following year suggests that the hybrid mortality in 1951 was due, in large part, to laboratory technique.

In 1952, two pairs that were cross-mated in nature were collected at the Federal Fish Hatchery and placed in enamel pans in the laboratory, at 11:30 P.M., on March 30. The eggs laid by each cross-mated female were undergoing early cleavages when examined at 8:00 o'clock the next morning. Samples were taken on April 2 (approximately 48 hours after ovulation) and, while most of the embryos at this time were in the early neurula stages of development, some had reached the late neurula stage (Rugh, 1948, Stage 17). Samples taken April 3 (approximately 72 hours after ovulation) were found to be in early tadpole development (Rugh, Stage 20, gill circulation). The tadpoles were all free swimming on April 4. Seven toads from this laboratory cross were raised to sexual maturity. A further check was made on this cross in 1953 when *woodhousei* eggs were artificially inseminated with *valliceps* sperm. Approximately 100 per cent of the eggs used in this cross were fertilized. No abnormalities were observed. An estimated 70 to 80 per cent of the zygotes developed

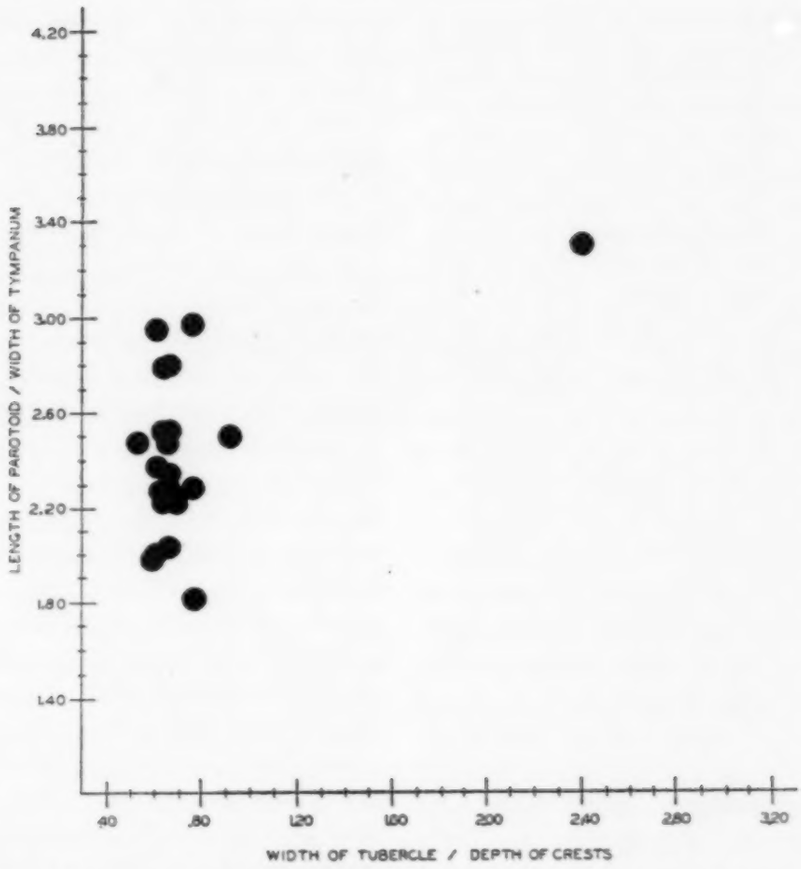

FIG. 6. Scatter diagram to estimate relationships in potentially hybridizing natural populations of *Bufo woodhousei* and *B. valliceps* toads. Dots represent individuals in a random sample of 24 toads (23 *valliceps*, one *woodhousei*) collected at the U. S. Federal Fish Hatchery on May 21, 1952.

through to the larval stage. The larvae were free swimming on April 11.

The results of these experiments indicate that viable hybrids can be produced by the crossing of female *woodhousei* with male *valliceps* toads. Whether or not gene exchange between the two species is possible depends, of course, on whether or not the hybrids are fertile.

CONTROLS

Woodhousei Female with *woodhousei* Male: As controls for the cross-matings in the laboratory, two pairs of *woodhousei* were collected from a roadside ditch in east Austin, on March 29, 1952, at 10:00 P.M. The eggs produced were undergoing cleavage when examined at 8:00 o'clock the following morning. On April 2 (approximately 72 hours after ovulation) the eggs were in the early tadpole stages of development (Rugh, 1948, Stage 20, gill circulation).

Valliceps Female with *valliceps* Male: As additional controls for the cross-matings in the laboratory, two pairs of *valliceps* were collected at the hatchery on March 30, 1952, at 11:00 P.M. The pairs were placed in breeding pans in the laboratory. Both females produced eggs which were fertilized by the clasping males. The zygotes were in the cleavage stages of development when examined on the following morning at 8:00 o'clock. The early tadpole stages of development were reached in approximately 72 hours from time of ovulation.

COMPARATIVE RATES OF DEVELOPMENT IN HYBRIDS AND CONTROLS

There appears to be very little difference between the hybrids and the normal

TABLE II. *Measurements in millimeters of length (head to tip of tail), and greatest width of* Bufo woodhousei, B. valliceps, *and hybrid larvae, raised under similar environmental conditions, in enamel laboratory pans.*

Samples preserved in five per cent formalin when larvae were 25 to 26 days old. First metamorphosis in days from fertilization.

Larvae	Measurements				First metamorphosis (days)
	Number individuals	Age (days)	Width	Length	
Hybrid	5	25	5–7	20–25	39
woodhousei	7	26	3–4	12–13	49
valliceps	6	25	3–4	15–16	49

zygotes of the two species in time of embryological development. In the laboratory experiments, the development from fertilized egg to the early tadpole stage, in each case, took approximately 72 hours.

The tadpoles produced in the cross and control matings on March 30–31, 1952, were separated on April 4, into groups of 30 individuals. Small random samples of the larvae (*woodhousei, valliceps,* and hybrid) were taken on April 24, when the animals were 25 to 26 days old, and preserved in five per cent formalin. Table II shows the measurements in millimeters of length (head to tip of tail), and greatest width of these animals, and the earliest date of metamorphosis for each of the three groups. The hybrid larvae were generally larger and metamorphosed earlier than either the *woodhousei* or *valliceps* controls. The more rapid development on the part of the hybrids suggests heterosis.

LABORATORY BACKCROSSES OF NATURAL AND LABORATORY HYBRIDS

Two of the laboratory-produced hybrids had developed the secondary sex characters typical of sexual maturity in toads by March 21, 1953. The two animals measured 60 mm. and 65 mm., respectively, at this time. Experiments were possible, therefore, to test the fertility of the hybrids through backcrosses to the parental species. Fortunately, one probable natural hybrid was collected on March 25, 1953, at the Federal Fish Hatchery. The somewhat peculiar but *valliceps*-like call of this animal was heard in a mixed chorus of *woodhousei* and *valliceps* toads. The toad showed a strong clasping reflex when collected and had the secondary sex characters well developed. The two laboratory hybrids, and the probable natural hybrid were used to attempt backcrosses to parent species.

Attempted Natural Backcross of Hybrids to *woodhousei* Females: The two laboratory hybrids with well developed secondary sex characters, and two female *woodhousei,* collected in the hatchery ponds, were injected with a gonadotrophic hormone Antuitrin S) on March 26, 1953, and were then placed by pairs in breeding pans. Four hours later, the male hybrids were observed clasping the female toads. By 7:00 o'clock the following morning one of the female *woodhousei* had completed ovulation, but the hybrid male was still clasping. The second female *woodhousei* was in the process of ovulation, with the hybrid male still clasping. No evidence of cell division was observed in eggs examined one hour after ovulation. The egg batches were separated into several enamel pans containing pond water and water weed and were kept under observation for three days. No development was discernible at the end of this period. Apparently none of the eggs had been fertilized.

Backcross of Probable Natural Hybrid to Female *woodhousei:* The probable natural hybrid collected at the Federal Fish Hatchery on April 25, 1953, and a female *woodhousei* collected there on April 26, were placed together in a breeding pan at 10:30 P.M., April 26. No gonad stimulating hormone was used. The probable hybrid immediately clapsed the female toad. Ovulation occurred during the early morning hours of the following day. Approximately 80 per cent of the eggs examined at 6:30 P.M. were seen to be in early cleavage stages. Many of the zygotes

showed what appeared to be abnormal cleavage. Development ceased in the third to fourth cleavage stages.

Artificially Induced Backcross of Natural Hybrid to *woodhousei* Female

Sperm from the probable natural hybrid collected at the hatchery ponds on March 25 was used to artificially inseminate eggs from a female *woodhousei* at 10:00 A.M., March 31, 1953. The sperm from a male *woodhousei* were used to fertilize some of the eggs from the same female as a control measure. The *woodhousei* eggs were mechanically extruded into separate petri dishes one of which contained a sperm suspension from one testis of the probable hybrid, and the other a sperm suspension from the *woodhousei*.

About 90 per cent of the *woodhousei* eggs inseminated with sperm from the field-caught hybrid were fertilized. A high percentage of abnormal cleavage was noted. At 3:00 P.M., approximately five hours after fertilization, all development ceased when the zygotes were in the third to fourth cleavage stages (8–16 cells). In the control experiment (*woodhousei* eggs with *woodhousei* sperm) approximately 100 per cent of the eggs were fertilized. The control zygotes were in the muscular response stage of development forty-eight hours after fertilization. All were free swimming larvae within 72 to 90 hours after fertilization.

Artificially Induced Backcross of Laboratory Hybrid to Female *woodhousei* and *valliceps*

The eggs of a female *woodhousei* and those of a female *valliceps* were mechancally extruded and artificially inseminated with sperm from one of the laboratory-raised hybrids, on April 7, 1953. The sperm from a male *woodhousei* and from a male *valliceps* were used in control inseminations of eggs of the same female. Eggs extruded from the female *woodhousei* were added to each of two dishes containing the separate sperm suspensions in pond water. In the same manner, eggs from the *valliceps* female were added to two dishes containing separate sperm suspensions. In this manner, four combinations of egg and sperm were obtained. These were: (1) hybrid sperm with *valliceps* eggs; (2) hybrid sperm with *woodhousei* eggs; (3) *woodhousei* sperm with *woodhousei* eggs; (4) *valliceps* sperm with *valliceps* eggs. Two and one-half hours after insemination the eggs from the four combinations were checked for cleavage. Fertilization was found to have occurred in all four of the combinations.

Hybrid Male with *valliceps* Female: Approximately 80 per cent of the eggs in this backcross were fertilized. Some abnormalities were discernible in the 8–16 cell stages of cleavage. All development ceased in early blastula.

Hybrid Male with *woodhousei* Female: Approximately three per cent of the eggs in this backcross were fertilized. Some probable abnormalities were observed in the early cleavage stages. One zygote survived blastulation. Forty-eight hours after fertilization this animal had developed to the early tadpole stage (Rugh, 1948, Stage 18). Metamorphosis occurred approximately four weeks after fertilization on May 8, 1953.

Woodhousei Male with *woodhousei* Female: Approximately 100 per cent of the eggs from this control experiment were fertilized. Early larval stages were reached within 48 hours after fertilization. The larvae were free swimming on April 11.

Valliceps Male with *valliceps* Female: Approximately 80 per cent of the eggs from this control experiment were fertilized. Early larval stages were reached within 48 hours after fertilization and the larvae were free swimming on April 11.

Description of Hybrids

Gonads: The gonads removed from the sexually mature *woodhousei-valliceps* hybrid for use in the artificial insemination experiments were significantly unusual. Parts of both testes have been preserved

MORPHOLOGICAL CHARACTERS		CATALOGUE AND FIELD NUMBERS: LAB. HYBRIDS ♂ 5555	5556	5894	752	753	759	NATURAL HYBRIDS ♂ 15551	15552	15553	15554	15558	15559	5613	5851	5856	5871	5893	TYPICAL WOOD. ♂	TYPICAL VALL. ♂
	INTERORBITALS WIDE, SHALLOW GROOVE	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
CRESTS	INTERORBITALS WIDE, DEEP GROOVE																			x
	INTERORBITALS NARROW																		x	
	CANTHAL CRESTS PRESENT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
	CANTHAL CRESTS ABSENT																		x	
	CANTHAL CRESTS EXTENDING TO NARES							x		x		x	x		x	x				x
	CANTHAL CRESTS TOGETHER BEFORE NARES	x		x	x	x	x		x		x			x			x	x		
	CANTHAL CRESTS WEAK, TUBERCULATED	x	x	x	x	x	x	x					x			x				
	SUPRATYMPANIC CREST PRESENT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
	SUPRATYMPANIC CREST ABSENT																		x	
MAXILLARY GROOVE	PRONOUNCED																			x
	WEAK	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	ABSENT																		x	
TYMPANUM	VERTICAL	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
	OBLIQUE																			x
	TOUCHING SUPRATYMPANIC CREST	x	x					x				x	x	x						x
	FREE OR BARELY TOUCHING CREST			x	x	x			x	x	x				x	x	x	x		
DORSAL STRIPE	WIDE	x	x		x				x	x	x	x		x		x	x	x		x
	NARROW			x		x	x	x					x		x				x	
INNER METATARSAL TUBERCLE	WEDGE-SHAPED																		x	
	INTERMEDIATE	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	OVAL																			x
COLORATION	WOODHOUSEI - LIKE	x	x					x		x			x		x	x	x			
	VALLICEPS - LIKE			x	x	x	x		x		x	x		x				x		
VENTER	SPOTTED		x	x			x	x	x	x		x		x						
	CLEAR												x		x	x	x	x	x	x
	RETICULATE	x			x	x					x									
SNOUT	POINTED		x		x	x	x	x		x	x	x	x		x		x			x
	TRUNCATE																		x	
	INTERMEDIATE	x		x					x					x		x		x		
SECONDARY SEX. CHAR.	THROAT DARKLY PIGMENTED																		x	
	THROAT LIGHTLY PIGMENTED			x		x		x	x	x	x	x	x	x		x	x	x		x
	THUMB DARK			x	x	x	x	x	x		x	x		x	x	x	x	x	x	x

FIG. 7. A comparison of the morphological characters of six hybrids from the laboratory cross of female *woodhousei* with male *valliceps* and 11 probable hybrids recognized in nature, with similar characters found in the species *woodhousei* and *valliceps*. The presumed natural hybrids were all collected at the U. S. Federal Fish Hatchery in southeastern Austin.

in a fixative (Bouin) for future histological study. Under the dissecting microscope the hybrid gonad has the appearance of being composed of half ovarian and half testicular tissue. The presence of what would appear to be undeveloped eggs is clearly discernible. Nevertheless, the sperm suspension from this animal was used successfully to fertilize eggs in backcrosses that were attempted.

Only one testis removed from the probable natural hybrid was used in making a sperm suspension for backcross experiments. The other testis was sectioned and preserved in two different cytological fixatives by Dr. M. J. D. White, for future study. The testes in this individual were highly atypical in both shape and size compared with sexually mature testes in the species *woodhousei* and *valliceps*. The probable hybrid testes were globular, white in color, and granular in appearance.

The characters of the gonads and the external morphological characters of the hybrids both are of interest.

External Morphological Characters of Metamorphosed Toads: The morphological characters of six hybrids from the laboratory cross of female *woodhousei* with male *valliceps*, and 11 probable hybrids recognized in nature, are compared in figure 7, with similar characters found in the species *woodhousei* and *valliceps*. All of the toads examined are preserved in 50 per cent isopropyl alcohol, and catalogued in the Texas Natural History Collection. The presumed natural hybrids were collected at the Federal Fish Hatchery, in southeastern Austin, and have been discussed earlier.

All of these toads show male secondary sex characters (i.e., a darkening of the excrescenses on the thumbs, and a darkening of the throat), with the exception of two juvenile toads that died in the laboratory before reaching sexual maturity. The laboratory-raised hybrids range in size (snout-vent) from 47 mm. to 65 mm. The presumed natural hybrids range in size from 75 mm. to 85 mm.

In general appearance, all of the laboratory hybrids and the presumed natural hybrids resemble the species *valliceps*. No natural hybrids have been recognized which resemble the species *woodhousei*. The intermediacy of the morphological characters of the hybrids between those of the two parental species is apparent only upon careful examination. As shown in figure 7, three morphological characters appear to be consistently intermediate in all of the hybrids examined: (1) the width of the cranial crests between the eyes, with the resulting shallow valley between them; (2) the weak groove developed along the edge of each maxilla; (3) an inner metatarsal tubercle (spade) intermediate in shape between those of the two parental species.

The most striking morphological difference between the laboratory-raised hybrids and the parent species is apparent in the pigmentation of the venter. In all of the hybrids the venter is heavily spotted to reticulate. Sixty-seven young hybrid toads from two cross-matings which were being raised in the laboratory all showed heavily spotted venters. The belly surfaces of *woodhousei* and *valliceps* toads in the study area are typically immaculate, or have a single, median, dark, pectoral spot in the case of *woodhousei*. It is not uncommon, however, to find individuals of either species with moderately heavy or light pigment spots. The significance of this heavy pigmentation on the venters of hybrid animals is not known. Field observations of marked toads over a period of several years would suggest that pigmentation on the venters is lost or obscured with age.

Discussion

It is agreed by most authors (Mayr, 1942; W. F. Blair, 1951; and others) that natural hybrids between sympatric vertebrate species are relatively rare. Mayr (1942, p. 260) brings out the point of view that sympatric hybrids are found primarily in genera in which copulation is not preceded by pair formation and an "engagement period." In the toads *Bufo* fertilization is external, and there would appear to be no mating preference at the breeding ponds.

The results of experimental crosses in the family *Bufonidae* (A. P. Blair, 1942), and in the family *Ranidae* (Moore, 1946) indicate that closely related anuran species are at least partially interfertile. In the species complex *Bufo woodhousei, B. fowleri, B. americanus,* and *B. terrestris* A. P. Blair (1942) found that cross-pairs produced at least some larvae which developed through metamorphosis.

The sympatric species *woodhousei* and *valliceps* are not considered to be closely related on morphological evidence. Zygotes from the cross-mating of female *valliceps* with male *woodhousei* are inviable. The reciprocal cross has produced fertile eggs the majority of which have developed through metamorphosis. Backcrosses of hybrid males to females of both species indicate a high degree of zygote inviability. Only one zygote (from the backcross of hybrid male to *woodhousei* female) has developed through metamorphosis. The majority of the zygotes developed no further than the blastula stages. There is, then, a highly effective genetic block to the exchange of hereditary characters between the two species.

Any factors effecting the normal production of eggs during the breeding season of *woodhousei* and *valliceps* might constitute a serious threat to the survival of either species. Of the thousands of zygotes produced from one normal species mating, presumably only a small percentage survive to maturity. The production of large numbers of inviable zygotes,

or sterile hybrids through cross-matings could theoretically reduce the size of the breeding populations of either species. The high percentage of cross-mating (eight per cent) found in the hatchery ponds would presumably be even higher if it were not for other isolation mechanisms which have been studied (in manuscript) such as time of breeding, and partial breeding site preference which apparently reduce the chances of cross-mating between the two species. The fact that only five probable hybrids (one per cent) were recognized in random samples from the hatchery would suggest that selection is working against the hybrid animals.

The egg complement of a single female *woodhousei* which laid in captivity was 25,644 by actual count (H. M. Smith, 1934). Egg production in the species *valliceps* is generally estimated to be between 10 to 15 thousand. The possibility that the high reproductive potential of these species nullifies the adverse effect of interbreeding and the consequent production of sterile hybrids cannot be completely ruled out. The potential offspring of a single female toad, if all survived, would comprise more than 100 times the individuals in a breeding population, such as that at the fish hatchery.

Summary

From a genetic standpoint, the relationships of the two species of toads may be summarized as follows: (1) male *valliceps* will cross with female *woodhousei* and at least some of the F1 generation males reach sexual maturity and produce viable sperm; (2) the reciprocal cross results in inviable offspring which die in late neurula stages of development; (3) the F1 males of the cross between *valliceps* male and *woodhousei* female produce mostly inviable zygotes when backcrossed to females of the parent species (the only exception is one metamorphosed young toad from the backcross to *woodhousei*). Most of the zygotes die at or before the blastula stage in development.

If no F1 females survive then the genetic block is very nearly or quite complete. If females survive, then they will have to be tested before one can be certain. The evidence now available suggests that there is little possibility of gene exchange between the two species, because of zygote inviability in one of the original crosses and in backcrosses to the parent species.

Acknowledgments

The experiments described in this paper comprise only a small part of a broad problem dealing with anuran ecology, which covers a period of four years. I am sincerely grateful to Dr. W. Frank Blair for originally suggesting the idea for this study, and for his continued guidance and encouragement during the course of the research.

I wish also to thank Hague L. Lindsay, Jr. for his help both in the laboratory experiments and in the field collecting. Parke, Davis and Company were most kind in donating a supply of gonadotrophic hormone (Antuitrin S) for use in laboratory experiments.

I am grateful to Dr. W. K. Clark for helpful suggestions in the preparation of the manuscript.

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MATING CALL AND STAGE OF SPECIATION IN THE *MICROHYLA OLIVACEA—M. CAROLINENSIS* COMPLEX [1]

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Introduction

This report deals with variations in mating call in a species group of narrow-mouthed frogs (genus *Microhyla*) [2] and with the possible evolutionary significance of these variations. Anurans were studied because differences in mating call serve as important isolation mechanisms in these animals. The particular species complex was selected because it has populations which appear to be in intermediate stages of speciation.

A western, xeric-adapted population that has been called *Microhyla olivacea* is distributed from the Gulf of California northeastward to eastern Texas and Oklahoma and northwestern Missouri. An eastern, mesic-adapted population that has been called *M. carolinensis* is distributed through the southern half of the eastern United States and reaches westward to eastern Texas and Oklahoma. The ranges of the two populations overlap in a strip running from the Gulf of Mexico into northeastern Oklahoma (fig. 1). Hecht and Matalas (1946) reported morphological intermediates between the unmottled *olivacea* and the dorsally and ventrally mottled *carolinensis* from a station in the overlap zone in southern Texas and from another in eastern Oklahoma. They hypothesized that the two are either approaching complete isolation or that previously existing isolating mechanisms have broken down. Their treatment of the two populations as subspecies has been followed by most anuran taxonomists.

The present study was undertaken in an effort to determine just how the two populations were behaving toward each other in the overlap zone. One obvious possibility would be that isolation mechanisms were breaking down and that the two populations would be bound together in future evolution by gene flow between them. Another possibility would be that as hypothesized by Dobzhansky (1937, 1940) limited hybridization and selection were acting to reinforce incipient isolation mechanisms. If the latter were true the two populations would be trending toward eventual elimination of gene exchange. Measurements of call inside and outside of the overlap zone should provide critical information about the behavior of the two populations toward each other. This is true because call differences are important isolation mechanisms in most anurans. The males go to breeding pools and attract the females by their calls.

Materials and Methods

Tape recordings have been made of the calls of 222 individuals at a total of 46 stations. The distribution of the recording stations is shown in figure 1 except for one station in southern Arizona and one in northern Florida. The distribution of the individuals in respect to the overlap zone is shown in table 1. All recordings were made in natural breeding aggregations. Recording equipment included a Magnecorder PT6 recorder and preamplifier powered by a storage battery and rotary converter with frequency controlled at 60 cps. An Altec dynamic

[1] This work was supported under National Science Foundation project No. NSF-G328.

[2] The generic name *Gastrophryne* has been resurrected from synonymy by de Carvalho (1954) to separate these frogs from the Asiatic genus *Microhyla*. The generic name has no bearing on the present problem, and I have followed Parker (1934) in retaining use of the name *Microhyla*.

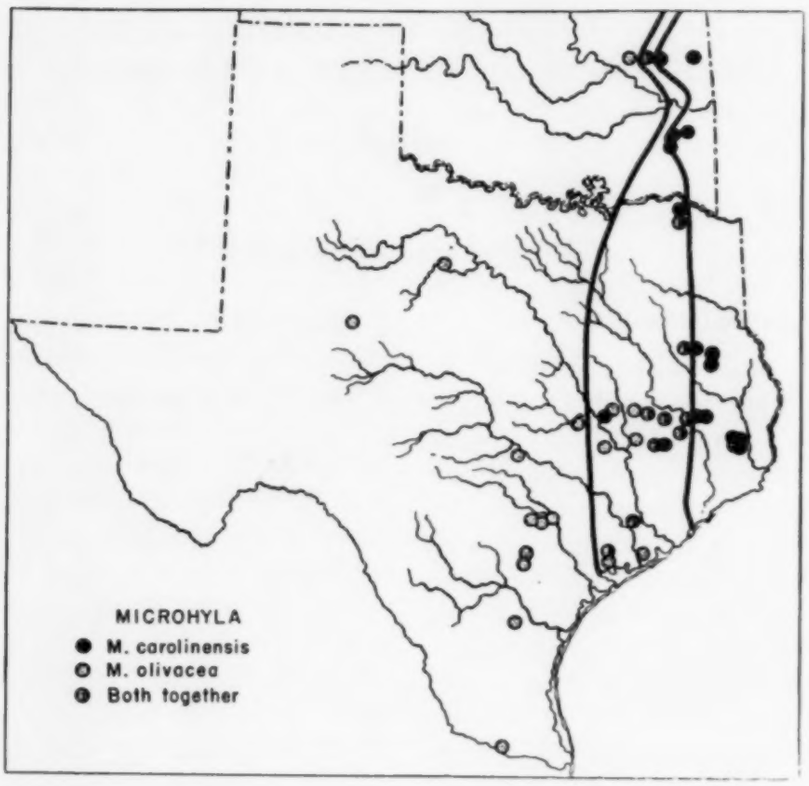

FIG. 1. Map showing approximate boundaries of overlap zone of *Microhyla carolinensis* and *M. olivacea*. Symbols represent stations at which recordings were made. The symbol "both together" is used for the three stations at which both species were recorded, the four at which one species and hybrids were recorded, and the three at which one species was recorded and the other was known to be present but could not be recorded for technical reasons.

microphone No. 660B was used with either a 100 or 200 foot cord. The microphone was placed close to the individual to be recorded in order to reduce background noise. All recordings were made at a tape speed of 15 inches per second. Air temperature near the ground and water temperature near the surface in the breeding pools were recorded. An attempt was made to collect the individuals recorded, but this was often unsuccessful with these tiny animals when certain types of vegetation filled the breeding pools.

In the laboratory, sound spectrograms of each individual call were made by use of a "Sona-Graph." With this instrument, the signal to be analyzed is recorded on a magnetic disc and then played back repeatedly into a scanning filter, the pass band of which is moved slowly across the frequency spectrum from 0 to 8,000 cycles. The output of the analyzing filter is recorded through a stylus on electrically sensitive paper that is fixed to a drum which rotates synchronously with the magnetic disc. The "sonagram" produced on the recording paper is a graphic representation of the call in respect to time, frequency and intensity. Time is shown on the horizontal scale. Frequency up to 8,000 cps is shown on the vertical scale. The intensity of marking indicates the amount of energy involved at each frequency level. All analyses were made with narrow band filters having an effective band width of 45 cps.

CHARACTERISTICS OF THE CALL

The call in the two kinds of *Microhyla* studied is a simple buzz. As shown by the sonagrams (fig. 2), the call consists of a fundamental vibration and a large series of up to 40 or more harmonics. Certain harmonics or bands of harmonics are weakly expressed; others are stressed, as indicated by their heavy marking on the

sonagram. The relative expression of the various harmonics and bands of harmonics is presumably a function of the vocal pouch. A few harmonic elements extend above the 8 kilocycle limit of the method used here, but the great bulk of the call and probably all of its significant parts fall within the limits of the present analysis.

Duration and frequency are the two most obvious variables in this kind of call. Duration has been measured from the sonagrams, where possible, by use of a plastic overlay scale calibrated in tenths of seconds. Where the call exceeded 2.4 seconds and therefore required the making of two sonagrams, the duration was determined from the tape recording by use of a stop watch calibrated to .10 seconds.

The best expression of frequency for the purposes of this comparison seems to be the mid-point of the emphasized band of harmonics. This usually falls between three and four kilocycles (fig. 2). It will be referred to as the emphasized frequency or mid-point frequency.

A comparison of frequency in the different samples must take into account the influence of temperature on frequency. Since these frogs almost always call from a partially submerged position, water temperature has been plotted against mid-point frequency to test the relationship between temperature and frequency (fig. 3). Separate regression lines have been plotted for the Texas-Oklahoma *olivacea* and *carolinensis,* using the method of least squares. Eight apparent field hybrids have been plotted on the scatter diagram, but they have not been used in calculation of the regression lines. Frequency is significantly correlated with temperature in both species. The correlation coefficient in *olivacea* is .883, and in *carolinensis* it is .273. The two regressions are obviously different; using the test of significance of Simpson and Roe (1939, p. 278) the probability that the two regression coefficients are the same is less than .0001.

Both the raw frequency data and the same data corrected to 25° C. are presented in table 1; in all other comparisons

TABLE 1. *Means and standard errors of duration and mid-point of emphasized frequency band in* Microhyla *calls*

Samples	Number of stations	Number of individuals	Mid-point of emphasized band in CPS		Duration in seconds
			Uncorrected	Corrected to 25° C	
Carolinensis					
Florida (Welaka, Putnam Co.)	1	26	3531±28	3484±30	1.25±.04
East of overlap (Texas, Oklahoma)	14	44	3009±34	2968±29	1.31±.06
From overlap zone (Texas, Oklahoma)	10	36	3070±43	3060±41	1.14±.05
Hybrids					
Overlap zone (Texas)	5	8	3599±61	3575±43*	1.52±.11
Olivacea					
From overlap zone (Texas, Oklahoma)	12	56	4403±40	4394±31	2.15±.05
West of overlap (Texas, Oklahoma)	11	50	3974±50	4141±40	2.45±.08
Arizona (Pena Blanca Spring)	1	2	3352	3564	1.45

* Average of correction factor for two species used.

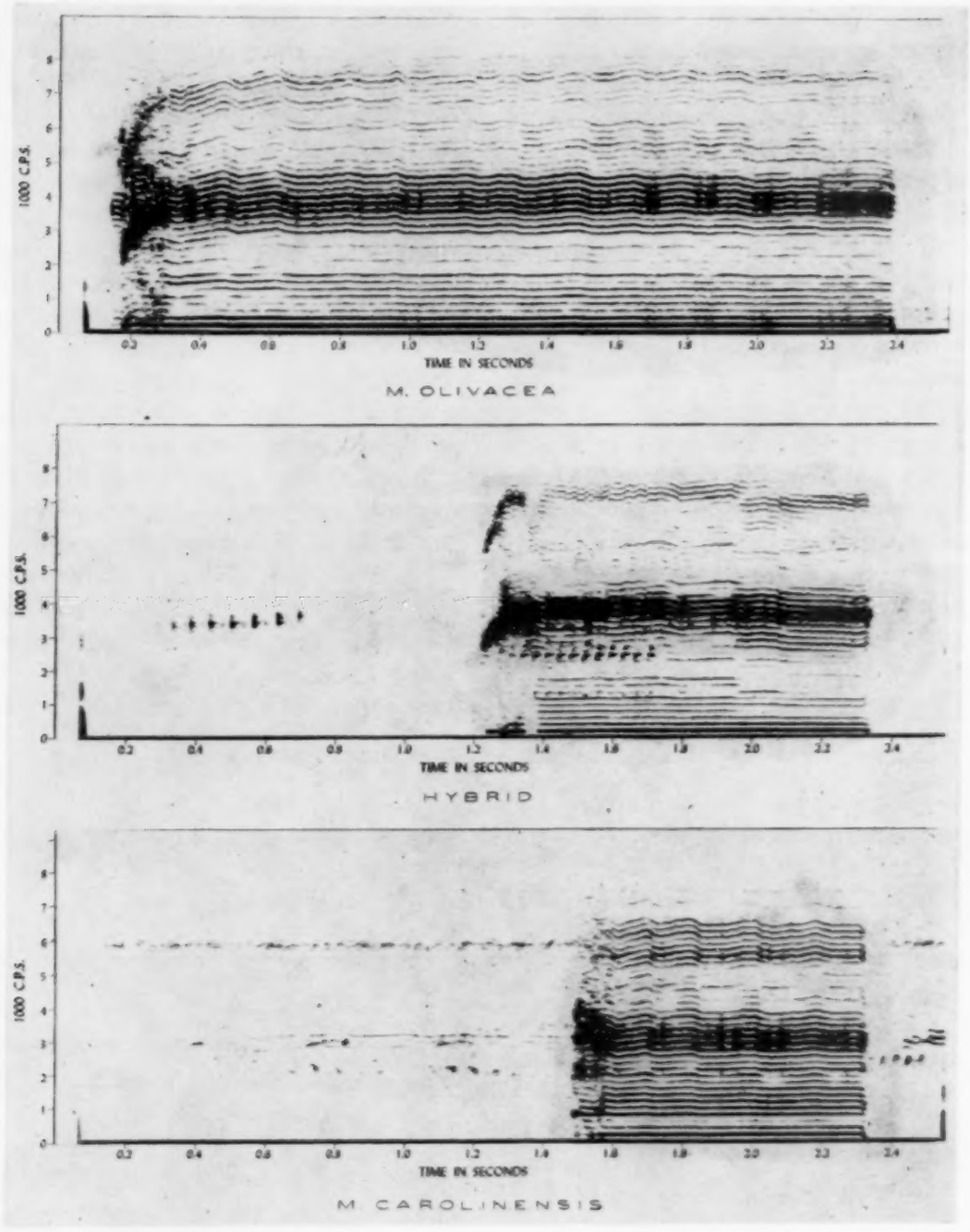

FIG. 2. Sonagrams of representative *Microhyla* calls from eastern Texas. Intensity of marking is proportional to energy involved at the frequency level. Note large number of harmonics, and emphasized band at about 3,000 cps in *M. carolinensis* and 4,000 cps in *M. olivacea*. Note differences in duration and note initial peep in *olivacea* and hybrid. Faint markings to left of call in hybrid and to left and right in *carolinensis* represent background noises.

the raw frequency data are used, because all differences between samples which are statistically significant in the raw data are also significant when the corrected frequencies are used. It is pertinent to the effectiveness of frequency difference as an isolation mechanism that the difference would be maintained in a mixed population and that it would actually increase as temperature increased.

In some cases there is a sharp rise in frequency at the start of the call. This is heard as a distinct peep. The presence or absence of this peep provides a significant

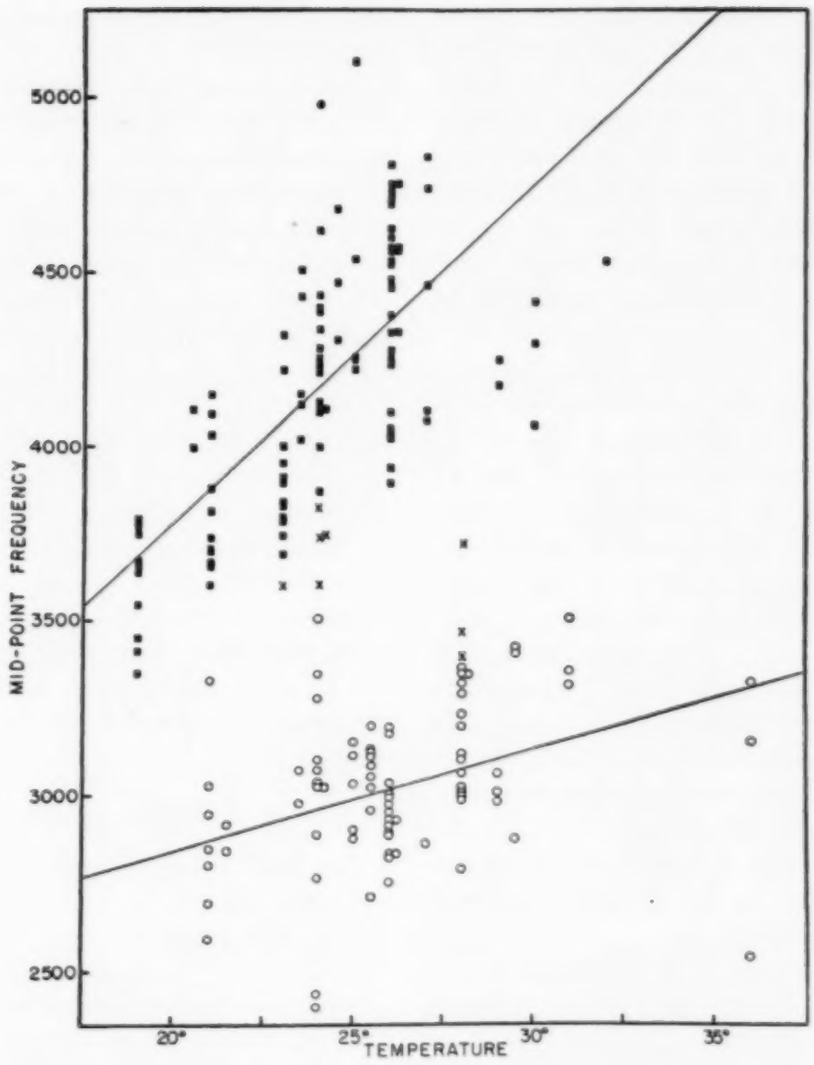

FIG. 3. Scatter diagrams showing relation between water temperature and mid-point frequency in *Microhyla*, with regression lines plotted by method of least squares. Solid squares represent Texas-Oklahoma *olivacea;* open circles show Texas-Oklahoma *carolinensis*. The symbol X shows presumed hybrids, which were not used in plotting regression lines.

qualitative character for comparison between populations.

COMPARISONS

The 222 individual calls available for analysis have been classified into seven groups (table 1). These are: (1) *olivacea* from the overlap zone in Texas and Oklahoma, (2) *carolinensis* from the overlap zone, (3) eight individuals from the overlap zone which were identified as probable hybrids on the basis of call and of morphological characters, (4) *olivacea* from west of the overlap zone in Texas and Oklahoma, (5) *carolinensis* from east of the overlap zone in Texas and Oklahoma, (6) two individuals of *olivacea* from southern Arizona (Peña Blanca Spring, Santa Cruz County) and about 800 miles west of the overlap zone, (7) *carolinensis* from northern Florida (Welaka, Putnam County) about 800 miles east of the overlap zone.

In the Overlap Zone

One hundred individuals were recorded in the overlap zone and at a total of 19 stations. Both species were present together at three stations. One or the other species was found in company with apparent hybrids at four other stations. The eastern species was found alone at four

stations. The western one was found alone at five localities, and at two others the eastern species was heard but could not be recorded for technical reasons. Absence of a species from a recording station does not necessarily mean that the species does not breed there. It means only that it was not calling there when the recordings were made or could not be recorded.

Disregarding the eight apparent hybrids for the moment, the remaining 92 individuals are clearly referable to one or the other of the two species. The call of *olivacea* is higher in pitch than that of *carolinensis;* the mid-point of the emphasized band averages 1,333 ± 58 cps higher in the former than in the latter species (table 1; fig. 4). The duration of the *olivacea* call averages nearly twice the length of the *carolinensis* call. The call of all of the 56 *olivacea* starts with a distinct peep which is seen on the sonagram as a sharp rise in frequency; the call in three of the 36 *carolinensis* has a slight initial inflection and that of the others is without evidence of the peep.

The eight presumed hybrids are intermediate between *olivacea* and *carolinensis* in both mid-point frequency and duration, although their means for both measurements fall somewhat closer to *carolinensis* than to *olivacea* (table 1; fig. 4). A slight to moderate peep is present in the call of six of the eight hybrids, and the other two show no initial rise in frequency.

Overlap with Non-overlap *olivacea*

Fifty *olivacea* were recorded at a total of 11 stations located from 50 to 300 miles west of the overlap zone in Texas and Oklahoma. The call of these western *olivacea* averages significantly lower in frequency than the call of *olivacea* in the overlap zone; the mid-point of the emphasized band averages 529 ± 64 cps lower in the former than in the latter group. The length of the call averages 0.30 ± .06 seconds longer in the western than in the overlap-zone *olivacea*. There is a distinct peep in the initial part of the call of all of the 50 western *olivacea*.

Only two calls of *olivacea* could be obtained at the Arizona station, where this species is relatively rare. On the basis of these two individuals, the call of the *olivacea* living about 800 airline miles from the overlap zone is much lower in frequency and much shorter in duration than the call of *olivacea* both inside and outside the overlap zone in Texas and Oklahoma. In fact, the call of the Arizona *olivacea* resembles the call of *carolinensis* more than it does that of Texas and Oklahoma *olivacea*. The call of these Arizona *olivacea* seemed to be weaker in volume than that of any of the other *Microhyla* recorded, but this could not be measured with the equipment available. There is an initial peep in the call of the Arizona *olivacea,* but it is less distinct than in the Texas and Oklahoma *olivacea*.

Overlap with Non-overlap *carolinensis*

Forty-four *carolinensis* were recorded at a total of 14 stations located from five to 65 miles east of the overlap zone in Texas and Oklahoma. These average slightly but not significantly lower in frequency and longer in duration than do the *carolinensis* from the overlap zone (table 1; fig. 4). It should be noted that these *carolinensis* came from localities considerably nearer the overlap zone than did the *olivacea* from west of it. As in the overlap-zone frogs of the same species, the call of these *carolinensis* usually lacks the opening peep. Five of the 44 individuals showed a slight to moderate initial inflection.

Twenty-six *carolinensis* were recorded in northern Florida, about 800 airline miles from the overlap zone of the two species. The call of the Florida *carolinensis* is intermediate in frequency between the Texas-Oklahoma *carolinensis* and *olivacea* (table 1; fig. 4). In duration, the call of the Florida *carolinensis* does not differ significantly from that of the Texas-Oklahoma *carolinensis*. Four of the 26 Florida *carolinensis* show no initial

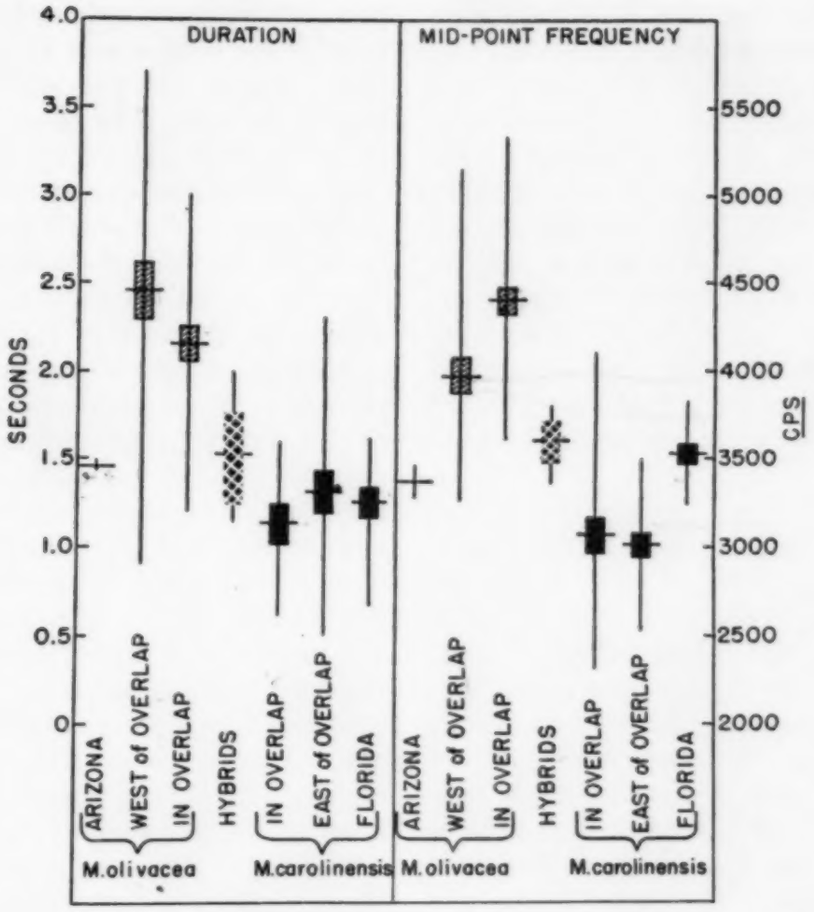

FIG. 4. Comparison of *Microhyla* calls by method of Dice and Leraas (1936). Vertical lines show range of variation; horizontal lines show mean. Failure of rectangles, representing twice the standard error on each side of mean, to overlap means that the difference between two means is at least four times the standard error of the difference. Note sharp differences between the two species in the overlap zone. Note intermediate position of presumed hybrids and of Arizona *olivacea* and Florida *carolinensis*.

rise in frequency. Three have pronounced rises, and the remainder have moderate ones.

DISCUSSION

Analysis of the mating calls leads to the following generalizations: (1) hybridization does occur in the overlap zone of *carolinensis* and *olivacea* as earlier reported by Hecht and Matalas (1946) on morphological evidence, (2) most overlap-zone individuals fall clearly into one species or the other, (3) calls of *olivacea* in the overlap zone differ more from those of *carolinensis* than do calls of *olivacea* from west of it; *carolinensis* from Florida are more like *olivacea* in call than are *carolinensis* from the overlap zone.

Distributional Relationships

Interpretation of the present relationships of *olivacea* and *carolinensis* in respect to gene interchange and speciational stage necessitates a reconstruction of the possible past history of the complex. It seems almost certain that the present contact of the two kinds is secondary as suspected by Hecht and Matalas (1946) and that it is post-Pleistocene. The general evidence for Pleistocene fragmentation of ranges in North America has been summarized by Deevey (1949) and that for the region and the species complex in question has been discussed by W. F. Blair (1951); this evidence does not need to be repeated here. There is no way of dating the contact beyond the strong cir-

cumstantial evidence that it is post-Wisconsin. There is evidence, however, that the present magnitude of the overlap zone has been influenced by clearing of the deciduous forest in this area. Except for the coastal prairie, the overlap zone roughly follows the western border of the eastern deciduous forest. Clearing for agricultural purposes along the forest border has resulted in the shift of terrestrial environmental conditions from humid toward xeric, and it has permitted the eastward spread of relatively xeric-adapted organisms.

The distributional pattern of the two species in the overlap zone strongly indicates a push eastward of *olivacea* into the cleared forest border. It also indicates a general withdrawal eastward of *carolinensis*. On the evidence from road logs of breeding choruses, the western species far outnumbers the eastern one in the overlap zone, and it is much more continuously distributed there. This is to be expected, as much of the area has been cleared of forest. The eastern species is discontinuously distributed in the overlap zone, and a few small disjunct populations exist in favorable localities west of what has been arbitrarily designated the western boundary of the overlap zone. One of these disjunct populations is in Gonzales County, Texas, in a peat bog known locally as Soefke's Swamp. A small collection of morphologically good *carolinensis* was taken there on April 24, 1949. Persistent efforts to locate *carolinensis* in Soefke's Swamp over the past two years have resulted in failure. Severe drought over the past five years has presumably depleted this population and may possibly have caused its extirpation. The species has never been found in apparently similar peat bogs in the same area nor in the mesic, post-climax deciduous forest of the flood-plain of the Guadalupe River there. Instead, enormous breeding congresses of *olivacea* have been found in rain pools on the heavily forested flood-plain. This is the only place in which we have found *olivacea* breeding in a situation which appeared ecologically more suitable for *carolinensis* than for it. One might argue that the small *carolinensis* population has been swamped through cross-breeding with the large *olivacea* population, but the mere existence of the small population of *carolinensis* until at least the spring of 1949 and the morphological distinctness of the specimens collected then make this explanation improbable. Depletion by drought seems the probable explanation of the apparent disappearance of this disjunct population of *carolinensis*. Another disjunct population has been reported to me by Ottys Sanders (oral communication) at a place locally called Bois d'arc Island on the Trinity River, just below Dallas, Texas. Blair and Laughlin (in press) have reported two apparently disjunct populations from mesic, flood-plain localities of the Verdigris River drainage in Wagoner and Tulsa counties, Oklahoma. The general distributional picture is one, then, of eastward expansion of the xeric-adapted *olivacea* into an area in which the eastward withdrawing *carolinensis* has left discontinuously distributed populations in favorable localities. These distributional changes were very possibly going on before disturbance of the forest border by man, and they have almost certainly been accelerated by clearing of the forest. The western, disjunct populations of *carolinensis* probably have been in contact with *olivacea* for a relatively long period of time, for they are in small areas of mesic environment well west of the historically known border of the deciduous forest and in which the regional environmental conditions have been relatively xeric for a long period.

The one place in the overlap zone where the distributional relationships of the two species could not have been affected by clearing of the forest is on the coastal prairie in Texas. This coastal strip is an area of relatively moist grassland with little or no water deficiency at any season (see map in Thornthwaite, 1948). Drainage is poor on the flat coastal prairie, and in many places irrigated rice fields provide

even more moist conditions than the undisturbed prairie. It is possible that *olivacea* ranges eastward on the coastal prairie because it is a grassland and that *carolinensis* occurs there because it is moist.

Evolutionary Status

The relationships of the different samples in respect to mating call are shown graphically in figure 5. The greater difference in mating call of the two kinds of frogs in the overlap zone, where there is some hybridization, than where the two do not occur together is possibly explained as the result of selection against hybridization. The possibility that incipient isolation mechanisms could be reinforced through selection has been put forth by Dobzhansky (1937, 1940). Koopman (1950) has obtained convincing experimental evidence by use of population cages of *Drosophila*.

Certain weak isolation mechanisms other than difference in mating call probably operate to reduce the chance of interbreeding of the two species of *Microhyla*. Bragg (1950) and others have pointed out that *olivacea* is dependent on rainfall for breeding, while *carolinensis* is not, and that the latter is a "summer" breeder, while *olivacea* breeds on rain from April to September. From our own observations of the past two years in Texas, these differences appear to be of minor importance as isolation mechanisms. The earliest record of *olivacea* breeding was April 9 and the latest was September 3. The earliest record for *carolinensis* was May 2, and the latest was September 1. The latest record of large choruses of *carolinensis* was July 31, when large numbers of both species were breeding following torrential rains. Thus, *olivacea* starts breeding earlier than *carolinensis*, but large numbers of both species may breed over a span of three of the five months in which he former normally breeds.

Water and air temperatures at the recording stations indicate only a slight preference of *carolinensis* for warmer temperatures. Water temperatures where *olivacea* was breeding ranged from 19.0 to 32.0° C. (mean, 24.9), and the air temperatures ranged from 14.0 to 28.0° C. (mean, 23.7). Water temperatures where *carolinensis* was breeding ranged from 21.0 to 36.0° C. (mean, 25.6), and air temperatures ranged from 19.5 to 29.0° C. (mean, 24.3). The air temperatures for *carolinensis* are exclusive of those for two stations at which this species was recorded in chorus in mid-afternoon. Air temperatures at those times were 30.0 and 33.0° C., respectively. The daytime breeding of *carolinensis*, pointed out by Bragg (1950) and others, would reduce the probability of cross-mating with *olivacea*, which apparently breeds only at night. Our observations indicate, however, that these daytime breeding choruses usually continue into the night. The breeding of *carolinensis* in the absence of rain also is an incomplete isolation mechanism, as large breeding aggregations of both species have been found following heavy rain from May 2 to July 31.

Laboratory evidence shows that the two species can be crossed, but it does not show conclusively whether or not the hybrids are equally as viable as the parent species. A. P. Blair (1950) made reciprocal crosses between the two species from Oklahoma and reported virtually 100 per cent cleavage and apparently normal larval development in four of five crosses involving female *carolinensis* and in one cross involving a female *olivacea*. In one cross involving a female *carolinensis* and in two in which *olivacea* eggs were squeezed into a suspension of *carolinensis* sperm fertilization was reported to be somewhat lower. None of the hybrids metamorphosed, and failure of any to survive beyond 90 days was attributed to faulty technique. This worker regarded the reluctance of *carolinensis* males to clasp smaller females of *olivacea* as an incomplete isolation mechanism. Two crosses between overlap-zone *carolinensis* females and *olivacea* males in my laboratory showed a low percentage of cleavage by comparison with approximately 100 per

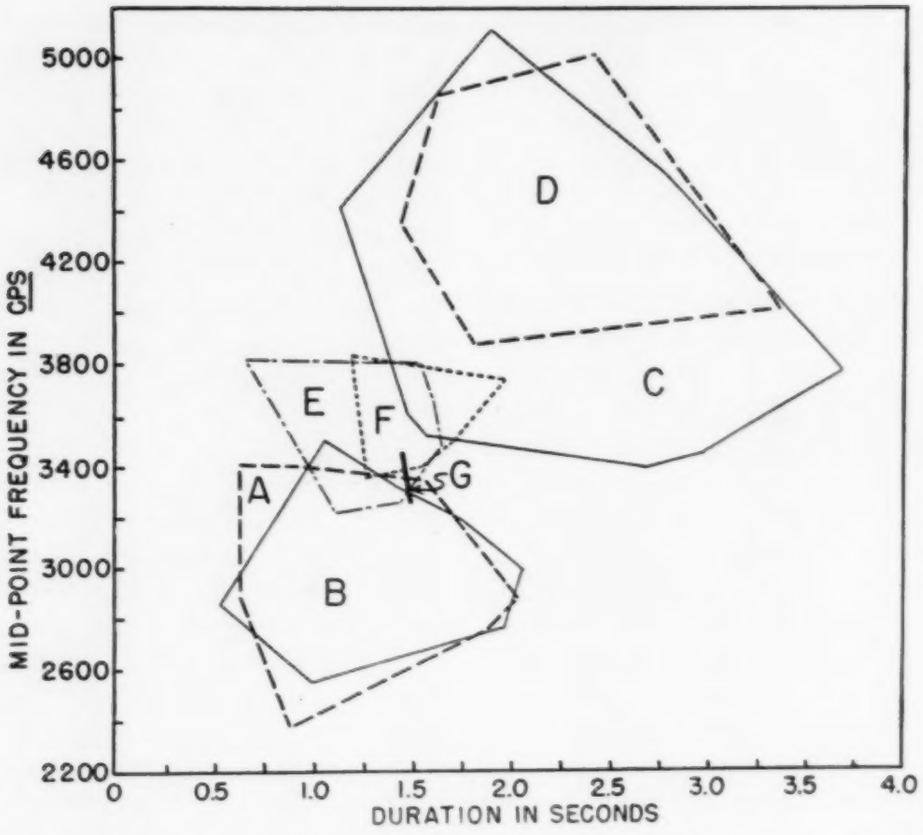

FIG. 5. Comparison of *Microhyla* calls by polygons constructed from scatter diagrams: (A) *carolinensis* from overlap zone, (B) Texas-Oklahoma *carolinensis* from east of overlap zone, (C) Texas-Oklahoma *olivacea* from west of overlap zone, (D) *olivacea* from overlap zone, (E) Florida *carolinensis*, (F) presumed hybrids from overlap zone, (G) Arizona *olivacea*.

cent cleavage in a control mating of *carolinensis*. The number of crosses is so small, however, that the lower development of the hybrids easily could have been due to condition of the eggs in females which had been in the laboratory for several days. A small number of both hybrids and controls metamorphosed and survived until they were about one-half to two-thirds grown, at which times they were lost apparently due to faulty technique.

Interbreeding of the two populations in the overlap zone is, then, restricted by a series of incomplete isolation mechanisms, most of which were presumably developed during geographic discontinuity of the two populations and as adaptations to different (xeric and mesic) environments. The total effect of the various mechanisms is insufficient, however, to prevent some cross-mating in the overlap zone. Perhaps the most significant isolation mechanism of all is difference in the mating call. If the calls are sufficiently different that females recognize and respond only to the calls of their own species, then the two species can use the same breeding pools and respond to the same meteorological conditions with only slight chance of cross-mating. The striking divergence in mating call in the overlap zone (figs. 4, 5) suggests selection against hybridization. If hybrids are at a relative disadvantage in competition with the parental species there should be greater survival of the offspring of males with the greatest call differences and of the females which respond to them. There should be relatively less survival of the offspring of males with minimal call differences and of the females which respond to them. Under such a system, differences in call which possibly originated at random during spatial discontinuity of

the populations would tend to be sharpened under present conditions of limited hybridization.

The argument for reinforcement of isolation mechanisms through selection against hybridization is premised on the hybrids being at a disadvantage in competition with the parental types. Direct evidence is not available, and it will be difficult, but not impossible, to obtain. The inconclusive results of laboratory hybridizations suggest but do not prove the possibility of reduced viability of the hybrids. The rather divergent behavioral adaptations of the two species to existence in contrasting xeric and mesic environments suggest that the hybrid genotype might be a disadaptive one. Morphologically, the relatively plain, olive-gray dorsum of *olivacea* provides good protective coloration in the muddy rain pools with scant vegetation other than grasses and sedges in which this species normally breeds and possibly at other times on the relatively vegetationless soils of its xeric environment. The dorsum of *carolinensis* is brown with blackish mottling, which makes it difficult to see against a background of dead leaves in the pools in which it normally breeds and at other times on the leaf-littered forest floor. The intermediate color and pattern of the hybrids would possibly provide relatively poor concealment on either type of background.

Judged from the evidence at hand, including characters of the mating call, the populations which have been called *Microhyla olivacea* and *M. carolinensis* are approaching final separation into separate breeding systems. If our interpretation of the evidence is correct, the relationships of these two populations are best represented by treating them as separate species.

Summary

Two species of narrow-mouthed frogs, *Microhyla olivacea* and *M. carolinensis*, have overlapping ranges in eastern Texas and Oklahoma. Limited hybridization occurs in the overlap zone. Contact of the two species is probably post-Pleistocene, and the present extent of the overlap appears to have been influenced by clearing of the forest.

The calls of 222 individuals have been compared by use of sound spectrograms in an effort to determine whether or not the isolation mechanism of call difference is being reinforced through selection against hybridization. In the overlap zone, the call of *olivacea* has a higher frequency as measured at the mid-point of the emphasized band of harmonics and is longer than the call of *carolinensis*. The calls of eight apparent hybrids are intermediate between those of the parent species in both frequency and duration. The call of *olivacea* from west of the overlap zone in Texas and Oklahoma averages significantly lower in mid-point frequency than that of *olivacea* from the overlap zone. The call of *carolinensis* from east of the overlap zone in Texas and Oklahoma does not differ significantly from that of overlap-zone *carolinensis*. The call of Arizona *olivacea* from about 800 miles west of the overlap zone is very similar to that of Florida *carolinensis* from about 800 miles east of the overlap, and both are intermediate in frequency and duration between the overlap-zone *olivacea* and *carolinensis*.

In addition to call difference, various weak isolation mechanisms affect interbreeding of the two species in the overlap zone. It seems likely that hybrids between the xeric-adapted *olivacea* and mesic-adapted *carolinensis* are at a disadvantage in competition with the parent species. Because of the role of call difference as an isolation mechanism, lower survival of offspring of males with minimal call differences and of females which respond to them would account for the markedly greater differences in call of the two species where they occur together than where they occur separately.

Acknowledgments

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NOTES AND COMMENTS

EVOLUTION AND OSCILLATIONS OF SHALLOW SHELF SEAS

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R. C. Moore (1954), in describing the evolution of several groups of invertebrates in rhythmic paralic sedimentation basins, postulates that this evolution is "in response" to the major oscillations of the shallow shelf seas, which caused the marine intercalations in the paralic sequence of strata.

The reason given for a causal relationship "oscillation of the sea → evolution" is that, during the transgressive phase of a sedimentary rhythm, areas for occupancy by marine organisms expanded vastly. With competition for food or for habitat easier during these transgressions, evolution might be accelerated theoretically. The condition for a genetic split-up in the expanding seas is, however, subsequent geographical isolation of part or parts of the populations. Only in this way may aberrant genetical characteristics, developed during a period of lessening competition, become fixed, to form starting points of new evolution.

Now it does not follow from the detailed work on rhythmic sedimentation that isolation of part of the invertebrate fauna has followed upon the transgressive phase of each or some sedimentary rhythms. Geological evidence seems even to point to the contrary. During a transgressive phase, the seas swept widely over the continental hinterland, which apparently had been base levelled into flat lowland plains. During the following period of regression, the sea withdrew bodily. Continental sedimentation took over on equally wide areas, without evidence of development of isolated islands and basins.

The very intensive work of R. C. Moore and his team of co-workers has given us detailed insight into the circumstances of rhythmic sedimentation. It follows that the numerous marine intercalations offer instantaneous pictures of the level reached by contemporaneous evolution. As such they are very valuable, a number of "stills" being easier to study in many respects than a running motion picture. They do not, however, offer any indication of how, where, and why evolution did arrive at these various levels at the time of the marine intercalations. First step towards a solution of these problems would be to compare the sequence of "stills" (the separate marine intercalations in a rhythmic paralic series) to the continuous picture (the sequence of fossil faunas found in the open ocean).

A factor marring all deductions from the populations fossilized in shallow shelf seas is that shallow seas often offer only a limited range in ecology, and consequently often only carry a part of the contemporaneous fauna. A striking example of this difference is found in the Upper Carboniferous of northwestern Europe and northern Africa. In the paralic sequence of the Westphalian in northwestern Europe, several cephalopods in the short-lived marine intercalations are found to be excellent index fossils. In northern Africa, the Lower Westphalian occurs in a normal marine facies. Here it contains throughout its sequence several forms which, in Europe, are restricted to definite marine intercalations. The most striking example is *Anthracoceras aegiranum,* which occurs throughout the Westphalian A and B in northern Africa, and is restricted to the Aegir marine intercalation that separates Westphalian B from Westphalian C in northwestern Europe (Deleau, 1951, 1952).

According to R. C. Moore, neo-zoologists are unable to study the effects of changes in life through enlargement or reduction of a given shallow sea. This is not true. All evidence gathered from polders or dry shallows, lost through storm or floods, points to the extreme rapidity of colonization. This can be measured in years or centuries. After this time, competition in the newly acquired habitats will be just as heavy as before. The process of colonization, according to the evidence of neozoologists, is very much quicker than the advance or retreat of a shallow shelf sea in rhythmic paralic sedimentation. Evidence of newcomers points the same way. For instance the pelecypod *Petricola pholadiformis* Lam. may be cited. It was imported from America to England around 1890, and has since successfully invaded the west coast of Europe (Benthem Jutting, 1943, pp. 278–280). A second example is *Mya arenaria* L. whose invasion began a little earlier, presumably around 1600 (Hessland, 1945). In these instances, humans were effective in the initial transportation, although this had no effect on the actual colonization process. This may be ruled out in the limnic pelycypod *Dreissena polymorpha* (Pal). This form is recorded from northwestern Europe

during the latest interglacial period. It retreated to southern Russia during the ultimate glaciation. From this area it has expanded in historical times, reaching for instance Holland only at the beginning of the 19th century (Benthem Jutting, 1943, p. 207). Contrary to the opinion expressed by Moore, neo-zoologists thus can indeed prove the extreme rapidity of colonization. Reduced selection pressure consequently is operative only during a short period, during a time wholly inadequate for speciation.

Theoretical conditions to be fulfilled if evolution really were "in response" to oscillations of shallow seas are as follows: (1) reduced selection pressure during transgression phases over periods long enough to initiate speciation. (2) isolation of aberrant parts of faunas considered. Neither of these two conditions has been indicated.

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EXPANSION AND CONTRACTION OF SHALLOW SEAS AS A CAUSAL FACTOR IN EVOLUTION

Raymond C. Moore

University of Kansas

The geologic record of past time shows on all continents the occurrence of widespread shallow-sea deposits which contain fossil remains of varied, more or less abundant benthonic invertebrates commonly associated with other organisms. As a rule, such deposits are separated from others higher or lower in the rock succession by unconformities which denote extensive exposure of the land to subaerial weathering and erosion. Patently, in the places where such unconformities are observed, sedimentation was interrupted during a relatively short or long part of geologic time, the region not being submerged then by shallow seas. In the same manner, the intercalation of areally widespread nonmarine deposits proves absence of shallow seas throughout the territory of continental sedimentation.

In late Paleozoic time, huge parts of both the Western Hemisphere and Old World were graded to nearly plane surfaces standing virtually at sea level in such manner that very slight rise of sea level would cause great inundation and similarly slight depression of mean sea level would cause very extensive emergence of the land surface. That shallow seas alternately transgressed the continental platforms and then retreated more or less completely into ocean basins is shown by the vertical succession and geographic distribution of cyclically arranged marine and nonmarine formations. This provides an exceptional opportunity for study of the effects of oscillatory movements of the shallow seas in relation to evolutionary changes of the marine invertebrates which have been preserved as fossils.

The fossil faunas of successive marine deposits which are separated from one another by nonmarine sediments, as found in late Paleozoic parts of the rock column, comprise composite records of shallow-water marine life which are not far removed in time in passing from one to another. Ecologic associations of identical type commonly can be found in each of the successive marine intercalations, and, by restricting attention to some one of these associations, comparison of congeneric species may be undertaken with the object of determining the nature and degree of changes presumably due to evolutionary modifications. This was attempted by me (Moore, 1954) in a paper to which reference is made in an accompanying discussion by M. G. Rutten. It is sufficient here to record the fact that several taxonomically distinct species belonging to some pelecypod and fusulinid (protozoan) genera now are described from Upper Pennsylvanian and Lower Permian marine strata of the northern mid-continent and Texas regions, providing suitable data for the study mentioned. They severally represent identical environments and hence do not reflect ecologic differences. The investigation

served to discriminate several seemingly definite evolutionary trends, but encountered the difficulty of distinguishing species belonging to an unbroken line of descent from species that may have been introduced by migration from some distant marine basin.

The concept that a major causal factor in evolution is the changing pattern of shallow seaways seems to be sound. As stated in the paper mentioned, transgressive shallow seas should provide easier competition for food and for places of attachment of bottom-dwelling organisms and permit existence of some species which could not endure rigorous competition. Adaptive changes, if they occur, should tend to become stabilized and little basis for postulating accelerated evolution is seen in times of expanding shallow seas. On the other hand, regression of a shallow seas inevitably produces crowding together of populations so that weaker, less well adapted marine invertebrates should be weeded out. "It seems reasonable to construe times of marine regression as more significant in terms of accelerated evolution than times of marine trangression" (Moore, 1954, p. 259). Unfortunately, no sure basis was found for recognizing successive links in an evolutionary chain and so of separating them from immigrant species having different ancestry.

The rapidity with which a species may migrate from one area to another obviously influences the distribution of organisms which become established in new territory during marine transgressions and it facilitates retreat during marine regressions, but this does not seem to be a significant factor affecting evolution. Thus, examples of rapid spread of marine species cited by Rutten (and others could be added) are irrelevant to inquiry concerning the influence of shallow-sea oscillations on evolution of ecologically restricted groups of invertebrates inhabiting parts of the shallow seas. Although geographic isolation of populations during retreat of a shallow sea certainly is possible, no appeal to this is made in arriving at the view that the crowding of formerly widespread benthonic invertebrates when these are pushed to margins of the continental shelf constitutes pressure that is mainly responsible for accelerated evolution at these periods.

Reference both to statements of this discussion and those given in the original paper cited (Moore, 1954) serves to show that Rutten's discussion is concerned mainly with attack on postulates introduced by himself, rather than set forward by me. For example, Rutten incorrectly attributes to me the concept that bottom-dwelling marine invertebrates can spread laterally only at geologically slow rates; then, on the basis of evidence that some species actually may colonize new territory very rapidly, he concludes that "reduced selection pressure consequently is operative only during a short period . . . wholly inadequate for speciation." Surely it is fallacious to assume that within a given stock differentiation of morphologically distinct populations (which are severally classifiable as species or subspecies) requires a long time or necessarily depends on dispersal accompanied by isolation. Other critical influences correlated with oscillatory shifting of shallow seas may be primary causal factors in evolutionary change, which also may be alternately retarded and much accelerated.

I may repeat the assertion that analysis of the evolutionary effects on organisms of shallow-sea transgressions and recessions can not be undertaken by the neozoologist. In order to obtain information comparable to that available to paleontologists, a zoologist studying the nature of evolutionary change of bottom-dwelling invertebrates in the Hudson Bay region or the Baltic-North Sea area, for example, would have to collect appropriate samples of these living populations and then arrange either for himself to survive a few scores of millenia or provide for supplementary investigations by some remote successor, so that if in the meantime these shallow seas should retreat to the margin of the continental shelf and then readvance, the new populations could be sampled for comparison with those now living. This sort of study is not possible. Only paleontological evidence can be employed for such comparisons. When analysis is confined to species belonging to identically the same habitat found in two or more successive marine bands which are separated by extensive nonmarine strata, and when immigrant species of "foreign" ancestry are eliminated, any observed morphological changes can be interpreted reasonably as originating from conditions which broad oscillation of the shallow-seas induced.

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THE MEASUREMENT OF VARIATION

J. B. S. HALDANE

University College, London

The coefficient of variation, that is to say the standard deviation divided by the mean, and usually multiplied by 100, is widely used as a measure of variation. This is fully justified, because the coefficients of variation are much more steady when we measure different organs in the same species, or homologous organs in different species, than either means, variances, or standard deviations. Bader's (1955) recent work on Oreodonts shows how valuable the coefficient of variation can be to paleontologists.

However the coefficient, as usually measured, has a defect which, fortunately, can be easily remedied. Some statistical parameters, such as the mean and the variance, have the useful property that an unbiased estimate of them can readily be found. The estimates of others are always biased. For example if we have n measments $x_1, x_2, \cdots x_r, \cdots x_n$, then $\frac{n\Sigma_r{}^2 - (\Sigma x_r)^2}{n(n-1)}$ is an unbiased estimate of the variance in the population from which the sample is taken. This means that if from a very large population (say all the male babies born in the U.S.A. in 1955) we take a series of random samples of n members, the expected value of the variance in a sample will be correct, and if we take N samples of n members, the average of the estimated variances will differ from the true value by a quantity tending to zero with $\frac{1}{\sqrt{N}}$. On the other hand the older formula $\frac{n\Sigma x_r{}^2 - (\Sigma x_r)^2}{n^2}$ has a bias. Its average is too small, being equal to the true value multiplied by a factor about $\left(1 - \frac{1}{n}\right)$. And if we took a large number N of such samples, their average would still be out by a quantity of the order of $\frac{1}{n}$, not $\frac{1}{\sqrt{N}}$.

The coefficient of variation is usually estimated by estimating the variance and mean of the whole population from a sample of n members, and dividing the square root of the estimate of the variance by the estimate of the mean. In what follows I use V for the true value of the coefficient of variation (expressed as a decimal, not a percentage), γ_1 for the measure of skewness $\mu_3\mu_2^{-\frac{3}{2}}$, and γ_2 for the measure of kurtosis $\beta_2 - 3$, or $\mu_4\mu_2^{-2} - 3$. γ_1 and γ_2 are zero in the case of a normal distribution. Haldane (1956) showed that the mean value of an estimate of the coefficient of variation made as above, is

$$V - \frac{V}{8n}(2 + \gamma_2 + 4V\gamma_1 - 8V^2) + 0(n^{-2}).$$

That is to say, there are further terms in which the divisor is n^2, the first being $\frac{1}{2n^2}$, so that they can usually be neglected. If the sample is drawn from a normal population this becomes

$$V\left(1 - \frac{1 - 4V^2}{4n}\right).$$

Even if the true value of V is as large as 0.1 (10%) which is rather high in paleontological material, we can take $\left(1 + \frac{1}{4n}\right)V'$ as a nearly unbiased estimate of V, where V' is the estimate obtained by the usual procedure.

Haldane (1956) showed that the mean value of the estimate of V^2 made by dividing the variance by the square of the mean is

$$V^2\left[1 + \frac{V(3V - 2\gamma_1)}{n} + 0(n^{-2})\right].$$

The bias is independent of γ_2, and is usually negligible if V is small. This estimate is much more satisfactory when, as in the case of animal behaviour, the distributions considered are far from normal. But for most paleontological data, multiplication by $1 + \frac{1}{4n}$ is quite satisfactory.

A few numerical examples will show the effect of the correction. Bader (1955) in his table 1 gives the coefficient of variation of muzzle width of *Brachycrus buwaldi* as .0386 ± .0111. This is based on a sample of $n = 5$. Multiplying by $\left(1 + \frac{1}{4n}\right)$ or 1.05, we get .0405. The correction is 17% of the standard error, and therefore seems worth while, as it could make a considerable difference in a test of significance. In his table 2 the mean value of V for the same species is raised from 5.56% to 5.71%. Such corrections can make a difference when such delicate comparisons as some of Bader's are concerned, though I do not think that they would alter any of his conclusions.

To sum up, the estimate of a coefficient of variation from a small sample tends to be too low. An extremely simple correction removes most of the bias.

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EDITOR'S NOTES

The rather long lapse between time of publication of an issue of EVOLUTION and receipt of reprints of the papers is a matter of constant concern to the authors. As a rule there is an interval of six to eight weeks involved. This matter has been taken up with the Press in the hope that the period might be shortened. No material reduction is, however, possible, because of the tight publication schedule that the Press maintains, and the problem of sufficient help in gathering and binding reprints. Thus we ask the indulgence of authors in this matter, and hope that the unavoidable delay will not cause any serious inconvenience.

In the Secretary's report in the September issue of EVOLUTION, Volume IX, it was noted that the elapsed time between receipt of articles and their publication was approaching an average of approximately twelve months. We are happy to report that prospects for the reduction of this time, perhaps to about eight months, are good. It has been possible to reduce the backlog of accepted manuscripts materially by increasing the size of the last two numbers, while doing only minor violence to the budget.

We have received the following announcement from Dr. Mont A. Cazier:

The American Museum of Natural History has announced the establishment of The Southwestern Research Station. It is located on the eastern slope of the Chiricahua Mountains, near Portal, Cochise County, in southeastern Arizona. The property is within the limits of the Coronado National Forest at an elevation of 5400 feet.

The station was established for the purpose of making available research facilities for scientists and students in all branches of science, who have problems that can be investigated through the utilization of the faunal, floral and geological features of the area. It will be open during the entire year.

It is operated by the American Museum of Natural History, Central Park West at 79th Street, New York 24, New York and under the direction of Dr. Mont A. Cazier, Chairman and Curator of the Department of Insects and Spiders, to whom all inquiries should be addressed. Anyone interested in the station should write to the above named individual for the booklet which gives the details of the operation and a general description of the area.

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